HEWER'S TEXTBOOK OF HISTOLOGY FOR MEDICAL STUDENTS

REVISED BY

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PREFACE TO THE FIRST EDITION

THIS text-book of Histology is the outcome of twenty-one years' experience of teaching medical students, and is written primarily for them. The minute structure of the tissues and organs of the human body is described, but details that are primarily of academic interest are omitted, and instead references are given for further information. Emphasis is laid throughout on the "physiological" appearances and their relation to function in contradistinction to a fixed, so-called "normal" state. The reactions of tissues to various conditions are briefly described in order to help the student to distinguish between variations of structures that fall within physiological limits and variations that fall outside these into the realm of histopathology.

The illustrations are of the kind that seem to be the most helpful to students when actually examining specimens, namely, photomicrographs to show the general structure under a low magnification, and diagrammatic drawings of the particular features of the minute structure more highly magnified. Such drawings can be made to include several focal planes, and are consequently found more useful as a rule than high power photomicrographs.

A short appendix includes a description of the principles and more important methods of histological treatment.

The illustrations are original, except where acknowledgment is made in the accompanying caption. The specimens from which the drawings and photographs are made are my own preparations, with the exception of a very few that are class specimens. I am much indebted to W. Lofts for his technical help. All the photomicrographs are untouched, and are the work of E. W. Gildersleeves.

I should like to express my appreciation of the help and encouragement that I have received from Professor W. C. Cullis and Professor M. F. Lucas Keene.

Finally, I wish to thank my publishers for their helpful consideration throughout.

EVELYN E. HEWER.

SOON after revising the fifth edition of this work Miss Hewer, having retired, asked the Publishers to become fully responsible for the revision of all future editions.

PREFACE TO THE NINTH (REVISED) EDITION

In this revised version of the ninth edition the general format has remained unchanged, emphasis being still on the inter-relationship between structure and function at both optical and ultrastructural levels. The opportunity has been taken to correct several minor errors, to replace some of the figures and to add magnification scales to the photomicrographs.

I am particularly grateful to my colleagues in the Anatomy department at Oxford for their continuing helpful discussions and for the provision of illustrative material.

S. BRADBURY

INTRODUCTION

THE *cell* is the unit of which all living organisms are built up and is also the unit of functional activity: a knowledge of cell structure is essential for an understanding of the functioning of living tissues. Such knowledge has been gained by the examination of either living cells or with dead cells that have been fixed, or preserved (see p. 13). Unicellular organisms can carry out all the activities necessary for their own life and reproduction. A multicellular organism begins existence as a single cell: as this cell divides and proliferates, differentiation gives rise to groups of cells, each group differing in structure from other groups and each group adapted for some special function. These groups are the *tissues*. During development the cells may become separated from one another by intercellular substance, so that the fully developed body consists of cells and intercellular substances functioning together as a whole. The various tissues are closely associated in the structure of *organs*, and when any organ reacts to any physiological or pathological conditions all or any of its constituent tissues may be involved in the changes. An appreciation of this fact is of fundamental importance in interpreting microscopical pictures.

In the following account cells are considered first, then tissues, and lastly organs. Animal tissues are dealt with, not plant tissues, and reference throughout is to the human organism unless the contrary is stated. It should be remembered always that the cells are living structures existing in a labile, semi-fluid condition and continually undergoing modification in response to changing environment: the appearance of a fixed and stained preparation when seen under the microscope must always be interpreted with this in mind. By fixation and staining it is possible to show many structures within cells that cannot be seen in the living condition. These structures are sometimes artifacts, although constant methods of treatment reduce the artificiality to a constant level for comparative purposes. On the other hand, structures may be invisible, not because they are not there, but because their refractive index or their physico-chemical constitution is almost identical to that of their surroundings.

References for more advanced and detailed study:

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CHAPTER 1

SOME TECHNIQUES USED IN THE STUDY OF CELLS AND TISSUES

Many new methods for the study of histology have been developed during the last fifteen or twenty years. Some of these have such a wide application and are producing such important and interesting results that it is necessary for the student to be acquainted at least with the principles underlying them. The following is a brief account of the more important among them.

A. Methods of Looking at Cells

(1) **Phase Contrast and Interference Microscopy.** The need to study living cells is becoming increasingly important and the conventional optical microscope has severe limitations in this respect, as indeed, has the human eye. Most living cells are virtually transparent objects whose constituent parts differ but little from one another in refractive index, so that there is hardly any visible contrast between them. Moreover, the refractive index of most living cells is very close to that of the aqueous media in which they are likely to be placed for microscopical examination. In consequence living cytoplasm appears structureless and empty to a large degree. This is, of course, one of the reasons why, in the past, so much use was made of cells that had been chemically treated (fixed) and coloured with various dyes, because this method revealed structures that could not be seen in the living cell. It was often open to question, however, whether such structures were artifacts of the technique rather than objects actually present. The great advantage of phase contrast microscopy is that it introduces contrast by optical means into unstained preparations of transparent objects e.g. living cells. A mathematical treatment of the theory of phase contrast is complex, but the following general account will serve to introduce the principle of the method.

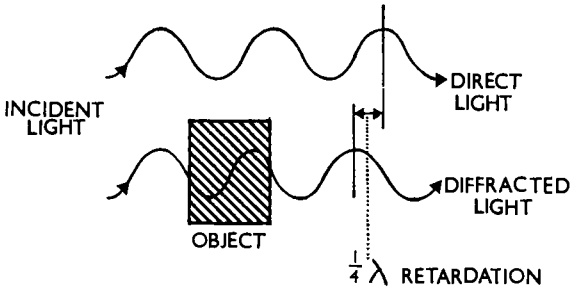
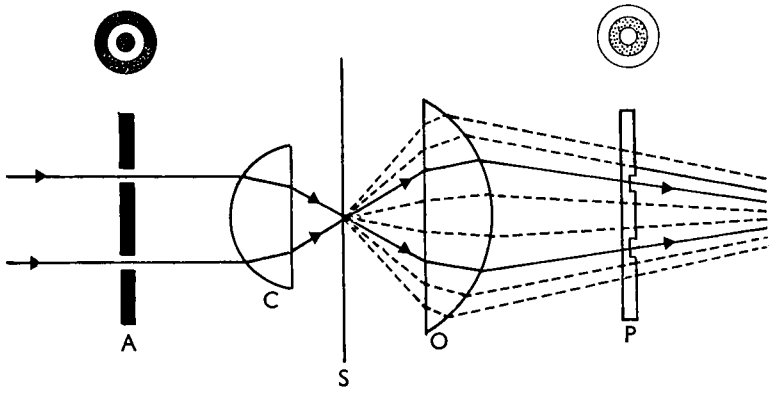


FIG. 1.1.—A diagram of the effect of a transparent object on light. The waves which have passed through it are retarded in phase by about ¼λ with respect to the original beam but their amplitude is unchanged.

When light passes through a transparent object which is mounted in a medium which differs in refractive index from itself, it does so with no change in the amplitude of the light waves. Since the retina of the eye is sensitive only to amplitude changes the object is therefore virtually invisible. Although there has been no change in the amplitude of the transmitted light it has nevertheless been affected by its passage through the object. Diffraction generates a set of new waves which differ in phase from the direct light which has not passed through the object (Fig. 1.1). This change in the phase of the diffracted light is of the order of a quarter of a wavelength (¼λ) for a typical cell.

The microscope image is formed as a result of interference between the direct light and the light diffracted by the object, so that if the phase difference between these two sets of waves can be further increased by optical means until the total difference between them is about $\frac{1}{2}\lambda$, then maximal destructive interference will result. There will thus be formed amplitude differences which can be recognized by the eye. The transparent object on the stage of the microscope thus is seen as if it were an absorbing object and it appears dark to a greater or lesser degree, depending on the amount of phase change which it has introduced into the diffracted light.



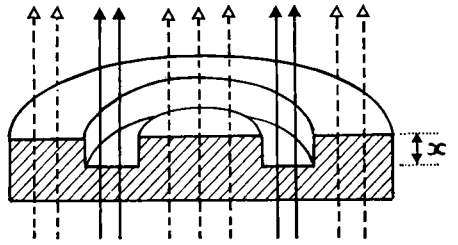


FIG. 1.2A (top).—The principle of the phase-contrast microscope; A represents the illuminating annulus, C the condenser, S the plane of the specimen, O the microscope objective lens and P the phase plate situated in the back focal plane of the objective. The path of the diffracted light is shown dotted, that of the direct light in a full line.

FIG. 1.2B (below).—A diagram of the phase plate in the objective. Note that the direct light all passes through the groove in the plate, whilst the diffracted light suffers a further retardation by having to pass through the extra thickness "x".

The practical problem, clearly, is to devise an optical system whereby the incident and diffracted waves are separated and the phase difference between them increased by $\frac{1}{4}\lambda$. This was achieved many years ago by Zernike but the significance of his discovery was not immediately appreciated by biologists.

Fig. 1.2A shows the optical system of the phase contrast microscope. An opaque diaphragm (A) containing a transparent annulus is placed at the back focal plane of the substage condenser (C). Direct light, unaffected by the object on the stage at S, will form an image of the annulus in the back focal plane of the objective (O); the diffracted light which appears to emanate from the object will, however, be evenly spread over the same area. Inside the objective a transparent phase plate (P) which has a circular groove in it is mounted so that the direct image of the annulus (A) coincides exactly with the groove.

The diffracted light will pass through the full thickness of the phase plate both inside and outside the groove, whilst the direct light passes through the thinner area occupied by the groove. The extra thickness of the glass (x in Fig. 1.2B) that the diffracted light has to traverse is so calculated that it introduces a further $\frac{1}{4}\lambda$ phase change relative to the direct light. The resulting destructive interference will produce a final image in which considerable detail can be seen (e.g. nucleus, chromosomes, mitochondria, lipid droplets, etc.). Fig. 1.3 shows the appearance of a living fibroblast visualized by phase contrast microscopy.

When a living cell is immersed in a medium whose refractive index is the same as its own it shows minimum contrast, that is it becomes invisible in the phase microscope. Now, many cells will survive quite well in osmotically-balanced aqueous solutions of proteins such as bovine plasma albumin and it is not difficult to find, by trial and error, that solution of known

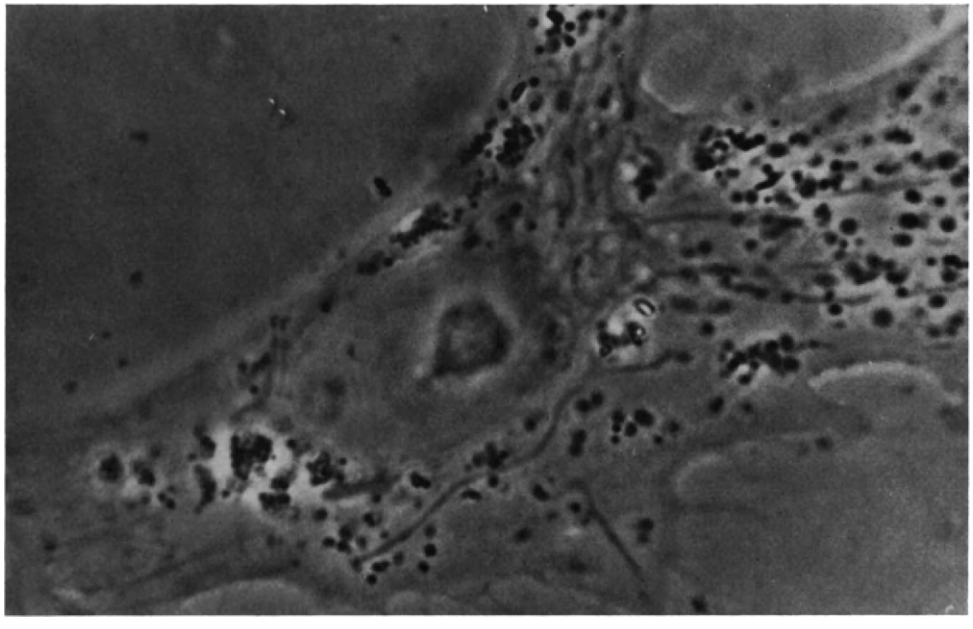


FIG. 1.3.—A living fibroblast in culture photographed with the phase contrast microscope. Note the "halo" around the cell border and the high contrast of the cytoplasmic inclusions. The mitochondria may be seen as long filamentous objects.

concentration which causes a particular cell (e.g. an erythrocyte, a fibroblast, etc.) to become virtually invisible when immersed in it.

Under these conditions the concentration of protein in the mounting medium will be approximately equal to that of the proteins in the cell. Since, in some cells, there may be significant amounts of other substances (e.g. carbohydrates) in addition to protein, more precisely the value obtained indicates the concentration of protoplasmic solids. Where this method of *cell refractometry* has been used the results have been in good agreement with those obtained by using other and quite different methods. Phase contrast microscopy has the great advantage that high contrast images of living cells can be achieved easily and with relatively simple equipment; because of the incomplete separation of direct and diffracted light, however, the image suffers from certain defects, especially from the presence of a marked "halo" around the edges where there is a sharp difference in refractive indices.

Like the phase contrast microscope, the *interference microscope* may be used qualitatively

to provide contrast in transparent objects and quantitatively to obtain information about the concentration of substances in cells and in addition to measure cell dry mass. In all types of interference microscope a beam of coherent light (i.e. light from a single source) is split in such a way that one part of the beam passes through the specimen and the other passes to the side

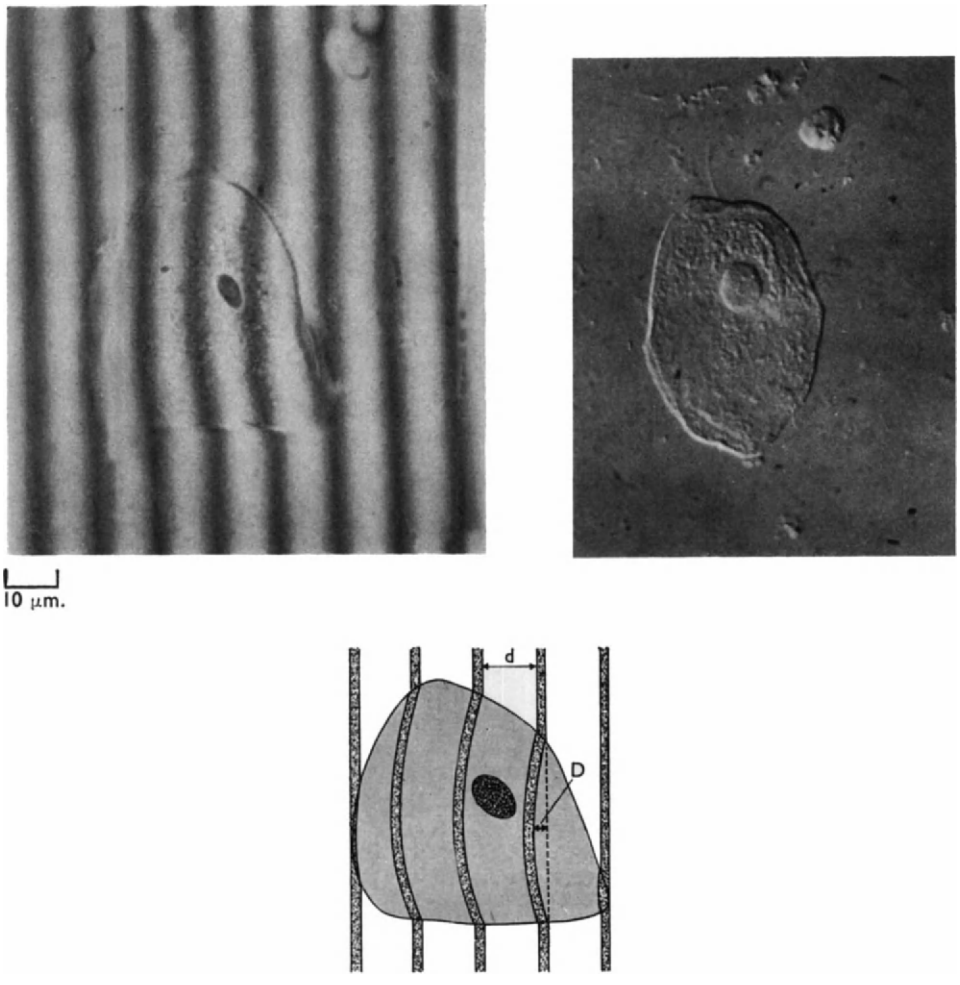


FIG. 1.4A (top left).—A buccal epithelial cell photographed with the Leitz interference microscope, arranged for fringe-field working; note how the bands are displaced where they pass through the cell. ×40 objective.

FIG. 1.4B (below).—A diagram of 1.4A to show how the displacement of the bands is measured and the value of D/d obtained.

FIG. 1.4C (top right).—A buccal epithelial cell photographed with the Nomarski interference microscope. Note the apparent "3D" effect.

of it. The light which bypasses the object is called the reference beam. Before entering the objective lens the two beams are recombined by a special system called a beam recombiner. One type of system is so arranged that the field of view has distributed across it a series of parallel dark interference bands, each band representing a zone of maximum destructive interference between the two beams at recombination. Where there is no object in the field the

interference bands are evenly spaced and are separated by intervals representing one wavelength (λ) (see Fig. 1.4A); when however a specimen, such as a cell, is introduced, the bands are displaced by varying amounts (see Fig. 1.4B). The degree of displacement can be measured at different points in the specimen and an average value obtained. If the displacement is D, and the distance between the bands outside the specimen is d, then $D/d\lambda$ (where λ for green light $= 5{,}460 \times 10^{-8}$ cm.) corresponds to a function known as the *optical path difference* or Δ. It can be shown that the dry mass (m) of an object $= \frac{\Delta . A}{100\alpha}$ gm. where A is the area in cm^2 and α is a constant, known as the specific refraction increment; m can therefore be calculated since the interference microscope gives the value of Δ, A can be measured by projection and α is known.

Recently interference microscopes have become available which use a principle of beam separation devised by Nomarski. Linearly polarized light is divided into reference and object beams by means of a double quartz wedge, the Wollaston prism, placed above the condenser. A second Wollaston prism above the microscope objective effects the recombination of the two beams. Images produced by a Nomarski interference microscope have a pseudo stereoscopic or "3-D" effect in addition to the usual interference colour contrast. The "3-D" effect is given by the appearance of a dark shadow on one side of refractile objects (Fig. 1.4C); structures of greater optical path difference than their surroundings appear to be higher and elevated above the background. Such an image may, on occasion, be of great value in interpreting the microanatomy of transparent objects.

(2) **Electron Microscopy (E/M).** In the electron microscope the energy source is a beam of electrons emitted from an electrically-heated tungsten cathode situated at the top end of a tube in which a vacuum is constantly maintained by means of a series of pumps. The beam of electrons is accelerated, by a high potential difference, towards the anode which has a circular aperture at its centre through which the electrons pass. Beneath the anode are placed, at appropriate intervals, a series of magnetic "lenses" which are, in fact, magnetic fields and correspond to the condenser, objective and eyepiece lenses of the conventional optical microscope. The object is placed between the condenser and the objective in the focal plane of the latter. The electron beam behaves in many ways like a beam of light and its path through the magnetic "lenses" is similar to that taken by light waves in the ordinary microscope. On passing through the specimen, which is usually a very thin section of fixed tissue, some electrons are absorbed while others are scattered. The transmitted electrons are finally focused to give a greatly enlarged image which is projected on to a fluorescent screen, since the eye is not sensitive to an electron image. The screen is used for scanning the specimen, but permanent records are made on photographic film or plates suitably placed inside the microscope.

The great advantage of the electron microscope lies in its far greater resolving power as compared with the optical microscope. The resolving power of the latter, that is its ability to image two small objects close together so that they appear separate and not as one, is a function both of the numerical aperture of the objective and of the wavelength of light; in practice, the minimum resolvable distance between two objects is approximately 0·2 μm. (μm. = micrometre $= 10^{-3}$ mm.). The wavelength of the electron beam in the E/M, however, is very much less, so that its resolving power, and hence its ability to reveal detail, is greatly increased—by at least 100 times. A consequence of this is that the photographic negatives obtained can be greatly enlarged to reveal an enormous amount of detail.

Because of the very poor penetrating power of electrons, very thin slices of tissue have to be used in electron microscopy. Small pieces of tissue are generally fixed in a 1 per cent solution of osmium tetroxide buffered to pH 7·4 or in solutions of aldehydes (in this case electron density is provided by treating the tissue with osmium tetroxide at a later stage in the processing). The tissues are dehydrated in a graded series of alcohols of increasing strength

before embedding in a plastic; many different resins are in current use, but the majority are epoxy resins such as "Araldite" or "Epon". Sections are cut about 0·05 μm. thick on a special microtome using glass or diamond knives and then mounted on a copper grid which is placed in the E/M.

The advantage of the E/M as a tool of histological research is undoubtedly its tremendous resolving power but it does suffer from some drawbacks:

(i) it is necessary to use very thin sections, which introduces problems of visualization of the structure in three dimensions;

(ii) living tissues cannot at present be examined, hence a "static" impression of the structure is emphasized;

(iii) it is difficult to sample adequately the range of structure presented by cells in a large heterogeneous organ.

(iv) no information on the chemical composition of tissues is provided by the standard E/M techniques.

(v) it is difficult to examine surfaces in the conventional E/M.

Efforts are being made to overcome these drawbacks; for further details the article by Wischnitzer listed in the references to this chapter should be consulted.

Much biological use is now being made of the scanning electron microscope for the study of the surfaces of cells, and of hard materials such as cartilage, bone and dentine. In the scanning E/M a beam of electrons, generated and focused much as in a conventional E/M, is scanned in a raster fashion over the surface of the specimen by varying the electrical currents in a pair of beam deflector coils placed in the microscope column. The specimen is placed at the lower end of the column. When the electron beam hits the surface of the object secondary electrons are emitted from its surface and collected by means of a positively-charged collector placed above and to one side of the specimen. The impact of these collected electrons on a phosphor causes flashes of light which stimulate a photomultiplier tube, the amplified output of which is used to modulate the display of a monitor cathode ray tube which is scanning in synchrony with the main exciting electron beam. A picture is thus built up of the morphology of the specimen surface. At the present time the resolution of the scanning E/M, in its standard commercial versions, is not equal to that of the transmission E/M. Nevertheless, very valuable information may be gained by the use of the scanning microscope especially since there it has a very great depth of field, can operate at low magnifications and can provide stereoscopic photographs very easily. An example of the use of this microscope in the study of red blood cells is shown in Fig. 4.3 (p. 48).

B. Qualitative and Quantitative Methods for the Determination of the Composition of Cells and Tissues

These methods may be divided roughly into physical and chemical techniques, but there is considerable overlap between the two groups.

(1) Physical Methods

(*a*) **Fluorescence microscopy.** Some constituents of cells, e.g. vitamin A and porphyrins, have a characteristic primary fluorescence in ultra-violet light (U.V.). The distribution of such fluorescent materials can therefore be studied by means of the fluorescence microscope. This instrument uses a light source such as the high pressure mercury arc which is rich in U.V.; those wavelengths in either the U.V. or the blue regions of the spectrum which are required for excitation of the specimen are isolated by means of suitable glass filters and focused by the substage condenser onto the slide: the exciting radiation is absorbed by the fluorescent substances and then the energy is re-emitted at longer wavelengths. As the fluorescence emitted

by the specimen consists of wavelengths in the visible regions of the spectrum, it may be examined and photographed without difficulty. Excess U.V. is prevented from reaching the eye by the use of a glass U.V. absorbing barrier filter which is fitted into the optical system of the microscope.

Currently little use is made of primary fluorescence of cell components; of much greater value is the practice of introducing so-called "secondary fluorescence" by treatment of the tissues with certain dyes known as "fluorochromes". These, although often not coloured in the visible regions of the spectrum, fluoresce vividly. Secondary fluorescence has the advantage of being much more intense than the primary form and by careful choice of fluorochromes very valuable diagnostic information may be obtained. Recently specific histochemical methods using fluorescent end-products have been developed. One good example of a fluorescent technique is the demonstration of biogenic amines (i.e. substances like 5 hydroxytryptamine 5HT, noradrenalin and adrenalin) by the technique of Falck and his associates. The method involves freezing and drying the tissue before treating it with hot formaldehyde vapour at about 70°C. The conditions of this treatment must be determined empirically. Afterwards the tissue is embedded in wax, sectioned and the sections mounted onto microscope slides. The wax is removed and the sections are then mounted in liquid paraffin for examination with a fluorescence microscope.

The formaldehyde treatment induces the amines to become strongly fluorescent and by using suitable exciting wavelengths and combinations of filters in the microscope it is possible to differentiate between the fluorescent end products from different amines. It is thus possible to review the distribution of, say, 5HT in the mast cells and compare it with the distribution of noradrenalin in the terminals of sympathetic nerve endings. An example of the visualization of adrenergic nerve terminals in this way is given in Fig. 13.15 (p. 188) which shows the innervation of the muscle layers of a small vessel in the iris diaphragm of a rat.

(*b*) **Scanning electron-probe microanalysis.** If a focused electron beam is allowed to impinge onto the surface of a specimen, characteristic X-rays are emitted. Each chemical element produces X-rays of a few specific wavelengths. If these can be detected, displayed and counted then we have a method which not only allows us to identify the chemical elements present in a specimen but also to get some idea of their location and their amount.

An electron beam is generated, focused onto the specimen and scanned regularly across its surface by means of the coils and magnetic lenses in the column of the instrument (Fig. 1.5).

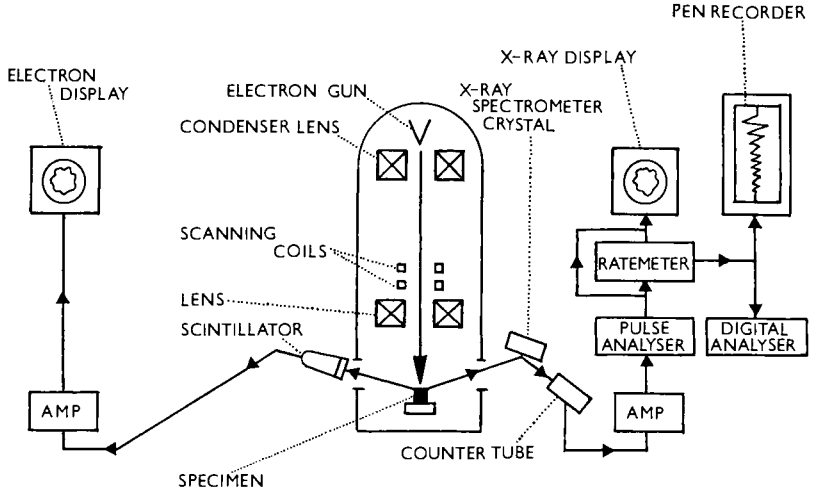


FIG. 1.5.—The components of the scanning electron-probe microanalyser (shown diagrammatically).

The X-rays which are emitted are collected and analysed by means of the X-ray crystal spectrometer. As the angle of the spectrometer crystal is altered, X-rays of the various different wavelengths may be isolated and passed into the gas-filled tube of the counter. The resulting signals are amplified and displayed on a cathode ray tube whose spot is scanning in synchrony with the original electron beam so that a picture of the distribution of that particular element is built up. In order to assist in the analysis a scintillation phosphor is also used to pick up the secondary electrons which are emitted from the specimen at the same time as the X-rays. This allows a scanning electron image to be built up, just as in the scanning electron microscope, so that a more detailed morphological picture may be obtained of the area that is emitting the X-rays. Often an optical microscope is also incorporated into the instrument so that a concurrent visual examination may be made.

Many refinements can be added to the instrument, such as the provision of a stationary probe of electrons, counting facilities, a pen recorder and the means of varying the crystal angle continuously. This latter allows a scan of a wide range of X-ray wavelengths to be carried out very easily so that the elements present may be rapidly recognized. As yet microprobe techniques are only just beginning to be used in biology. Studies have been made of the deposition of calcium in arterial walls for example, but once the technical problems involved in the preparation of biological material for examination in this instrument are overcome it is probable that much use will be made of this very precise technique.

(*c*) **Autoradiography.** The last physical technique to be described here has, in the past few years, become one of the most important tools of cytological and histological investigation. It is the method of *autoradiography* which enables the metabolic path of many radioactively labelled precursor substances to be traced in tissue sections. For example, the incorporation of iodine into the thyroid gland, the incorporation of amino-acids into intracellular proteins, and the incorporation of thymidine into DNA can be traced in this way.

It is now possible to obtain a large range of organic and inorganic substances in which one of the constituent atoms is replaced by a radioactive isotope. When such substances are made available to cells by injection into the whole organism or by other methods, they become rapidly incorporated. Tritiated thymidine (that is, thymidine containing ^3H or tritium), for instance, is rapidly taken up into all interphase nuclei which happen, at the time of the injection, to be synthesizing new DNA prior to nuclear division. Autoradiography is concerned with demonstrating the sites of such incorporation, by showing up their radioactivity.

The isotope atoms, being unstable, emit α particles, β particles or γ radiation according to their nature and all these products of atomic breakdown are capable of activating a photographic emulsion. Thus the essence of the technique is coating a layer of sensitive emulsion over the histological section or tissue smear and exposing it (in complete darkness) to the radioactivity for a suitable period of time, which has to be determined empirically. The overlying emulsion is then developed and fixed as if it were a photograph, when sites of radioactivity reveal themselves as clusters of black silver grains in an otherwise clear emulsion. The tissue can be stained either before the emulsion has been put on, or after it has been developed. It is thus possible to focus through the thin emulsion layer to the tissue underneath and discover what components have been the source of the radioactivity. These, of course, will be the sites where there has been active incorporation of the labelled substance. In the case of triated thymidine mentioned above, these will, in a successful experiment, be groups of silver grains, over a proportion of the nuclei. Providing the exposure times are carefully adjusted to the intensity of the emitted radiation, there should be very little "fogging" of the emulsion, so that a very exact localization of the radioactivity can be achieved.

It is often possible to estimate the amount of uptake of radioactive material by counting the number of silver grains which are visible above the objects of interest. Alternatively the autoradiographs may be examined in the microscope using incident rather than transmitted light. Under these conditions the silver grains reflect light which can be accurately measured

by means of a photometer attached to the microscope. This gives a rapid, semi-automated method of estimating the amount of radioactive incorporation into the tissue.

If tissue samples are taken at varying times after the initial injection of the labelled substance and autoradiographs made, it is possible to trace the movement of a labelled compound from one part of a tissue or cell to another. For example, labelled amino-acid is first demonstrable in the basal region of a pancreatic cell where the principal sites of protein synthesis are located. Sometime afterwards, the radioactivity is predominantly in the apical region where the enzyme precursor accumulates in the form of granules prior to its release to the exterior. The inference to be drawn from these observations is that enzyme synthesis first begins in the basal zone where some of the enzyme protein has labelled amino-acid incorporated into its molecular structure. Then, as the enzyme is transported to its final location at the apical pole of the cell, it naturally carries its radioactive constituents with it. From experiments of this kind, important information can be obtained about the movements of labelled cells in tissues and about the movements of labelled molecules within cells. Recently this principle has been extended to preparations intended for study with the electron microscope. The technique is essentially the same as for optical autoradiography; after incorporation of the isotope the tissue is fixed, dehydrated embedded and sections prepared as if for conventional electron microscopy. They are then covered with a thin coat of emulsion, usually applied in liquid form, and allowed to expose in the dark. Because of the very thin sections and their rather low levels of activity, exposure times tend to be longer than with material intended for optical microscopy. After development and fixation the grid, together with its overlying emulsion and silver grains, is examined in the E/M.

The resolution of this technique is much better than that obtained with the optical microscope; for example in the example quoted above (synthesis of protein in the pancreatic cell), it is possible with electron microscope autoradiography to recognize the label when it first appears in the rough surfaced endoplasmic reticulum, then in the region of the Golgi apparatus of the cell; finally after some hours it appears in the secretion granules at the apex of the cell. E/M autoradiography has proved a most valuable tool for the investigation of cellular function and it has helped to supplement the purely morphological observations which were hitherto all that was possible with the electron microscope with a new dynamic approach of great versatility.

It is, of course, of the utmost importance in experiments of this kind, that the dose of the radioactive substance should be such that there is no risk of the radioactivity interfering with the normal metabolism of the tissue being studied.

(2) **Chemical Methods.** In general, these methods aim to demonstrate the presence of important organic and other molecules within cells and tissues by means of specific chemical reactions. The operation is known as a cytochemical or histochemical test and *histochemistry*, as it is commonly understood, is a rapidly expanding branch of cell biology which is concerned with devising, evaluating and interpreting tests of this kind. As was pointed out earlier, however, there is considerable overlap between the physical and chemical methods of investigating the molecular structure of cells and tissues, and the techniques previously outlined will also be found included in textbooks of histochemistry.

Usually in histochemistry the test is applied to tissue sections which have already been chemically fixed. Sometimes, however, the tissue is fixed subsequent to the test. Clearly the choice of fixative used is extremely important, because although it must preserve the substance to be tested for, it must not react with it chemically or in any other way so as to alter those parts of the molecule which will be involved in the histochemical reaction. Thus, fixatives containing strong oxidizing or reducing substances are best avoided. It is held by many that the most satisfactory method of preparation is that of rapidly freezing small pieces of tissue at temperatures of −150°C and below, and then gradually removing the ice crystals either by evaporation in a high vacuum at a somewhat raised temperature or by dehydration in an

organic dehydrating agent (i.e. absolute ethanol or methanol) also kept at a moderately low temperature. Unless the temperatures are carefully chosen and controlled, however, there is the risk of considerable tissue damage resulting from the formation of large ice crystals. These techniques of *freeze-drying* and *freeze-substitution*, when successfully applied, have the following advantages: (i) they give good general preservation; (ii) freezing is so rapid that labile molecules have little chance to move from their true positions in the cell; (iii) there is minimal chemical interference; and (iv) enzymes lose less of their activity than after chemical fixation.

Apart from the demonstration of various metallic ions and inorganic radicals (e.g. phosphate), histochemistry is much concerned with the identification of proteins, carbohydrates, lipids, steroid substances and enzymes. A good histochemical reaction, however, should give information about the localization of a substance as well as its chemical identity. In this context it is clearly of paramount importance that neither the fixative, nor the chemical

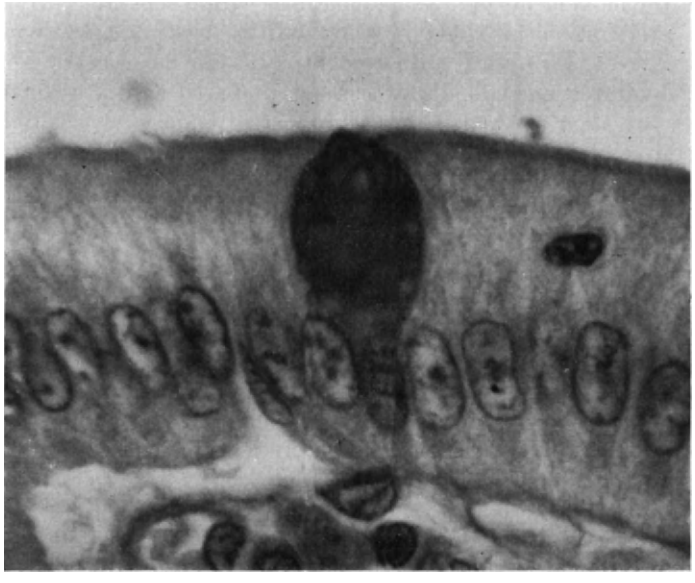


FIG. 1.6.—A goblet cell of the small intestine prepared by the PAS (Periodic Acid/Schiff) technique. Notice the dark staining of the mucin in the goblet cell.

procedures subsequently employed, should cause the substance to diffuse away from its naturally-occurring site in the cell or tissue. Substances known to be specifically located, for example, in nuclei, mitochondria, cell surfaces, etc., as the result of other types of investigation, must be shown to be similarly localized by the histochemical technique as well. The possibility of diffusion at some stage in the test still remains one of the great problems of histochemistry.

The general principles of histochemistry are best illustrated by examples and three such examples are given briefly here.

(*a*) *The PAS Reaction.* This reaction depends on the fact that certain polysaccharides such as glycogen and a number of proteins linked to carbohydrate (e.g. mucoproteins and mucopolysaccharides) yield an aldehyde after mild oxidation with periodic acid. This aldehyde can then be coupled with Schiff's reagent (a colourless addition compound formed by treating basic fuchsin with SO_2) to give a magenta-coloured reaction product. This reaction exemplifies certain principles common to many histochemical tests, namely: (1) the product of the primary chemical reaction is often colourless; (2) it does not diffuse significantly from its site of formation; (3) it is made visible by causing it to combine with another reagent to give a coloured

compound; and (4) this final coloured compound does not diffuse from its site of formation. In consequence, in the case of glycogen for example, it is reasonably certain that the magenta-coloured masses and granules seen in a liver cell represent the distribution of glycogen as it was in the fixed tissue. In this particular instance, an inaccurate picture of glycogen distribution is more likely to be caused by the process of fixation than by the subsequent chemical procedures. Fig. 1.6 shows the positive PAS reaction in the mucin of an intestinal goblet cell.

(*b*) *The Feulgen Reaction for DNA*. This again depends upon the production of an aldehyde and its coupling with Schiff's reagent. Here, however, the aldehyde is formed from the pentose sugar deoxyribose of the deoxyribose-nucleic acid by hydrolysis in N.HCl at 60°C. If this test is applied to nuclei in division there is a very precise localization of the magenta colour in the chromosomes.

(*c*) *The Gomori Method for Alkaline Phosphatases*. This method exemplifies most of the principles involved in the detection of enzymes in tissues. Firstly, maximal preservation of both the enzyme activity and the structural integrity is essential. This is best achieved by rapid freezing and then preparing sections of the tissue whilst it is still frozen in a cryostat. The sections, after mounting on coverslips, are processed without any chemical fixation. Secondly, a substrate medium closely resembling or identical with the normal one hydrolyzed by the enzyme *in vivo* must be used; tissue sections are incubated in this substrate at 37°C for a suitable time—empirically determined. Alkaline phosphatases act by hydrolysis at fairly high pH and in consequence the medium must be appropriately buffered. The substrate used is sodium glycerophosphate, from which phosphate is split off by the enzyme. The liberated phosphate is trapped by calcium ions previously added to the substrate. This sort of procedure, known as a "capture" reaction, is widely used in histochemistry and prevents the diffusion of a soluble reaction product by combining it with another ion at its site of production to produce a highly insoluble molecule. In this instance calcium phosphate is precipitated but, because it is colourless, the final operations in the technique are designed to convert it into a readily visualized but still insoluble compound. The first step is to place the section in cobalt nitrate, when the calcium is replaced, and cobalt phosphate is formed. The latter is still colourless, but a brief treatment with a dilute solution of ammonium sulphide converts it into black cobalt sulphide which is readily visible. Sites of enzyme activity consequently appear black in the finished preparation; kidney sections, for example, prepared in this way show an intense reaction in the brush border of the proximal convoluted tubules indicating a high concentration of the enzyme in this position.

In all histochemical procedures it is absolutely necessary to carry out control experiments. This is particularly the case when the reaction indicates only the presence of a class of substances rather than an individual compound. Thus, in the first example a positive reaction would only indicate glycogen if a similar section which had been previously digested with an amylase was negative to the PAS test. Similarly, a positive result in the Gomori test would only indicate the presence of an alkaline phosphatase if a similar section, in which the enzyme had been inactivated by heat or fluoride poisoning, produced a negative result. (A positive result after the latter treatment would probably indicate the presence of preformed phosphate.)

There are now very many histochemical and cytochemical tests in use, and descriptions of these will be found in books dealing specifically with the subject. The application of histochemical methods to electron microscopy is a field which is expanding rapidly at the present time.

In concluding this chapter brief reference must be made to a technique which, in combination with electron microscopy and biochemical analysis, has provided much important information about the distribution of chemical substances in cells. This is the method of *high-speed density gradient centrifugation*. Cell homogenates are spun at very high speeds after they have been placed in a tube on top of a series of fluid layers of carefully graded density; different cytoplasmic constituents tend to become separated into fairly distinct layers in the centrifuge

tube. Samples can be subjected both to direct chemical and biochemical analysis and to examination with the E/M. By this means correlations can be made between the biochemical activities of the various fractions and the structural elements characterizing them. Thus it can be shown that the catecholamines of the adrenal medulla are almost entirely localized to the large dense granules which occupy the cytoplasm of the medullary cells. Similar techniques have demonstrated that the enzymes of the Krebs cycle and the electron transport system predominate in the layer containing small cytoplasmic organelles called *mitochondria* (see Chapter 2) whilst acid hydrolases are probably confined to the *lysosomes*.

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CHAPTER 2

THE CELL AND CELL DIVISION

Free cells (e.g. leucocytes and ova) usually have a spherical or an ovoid shape; in most tissues, however, the shape of the cells becomes modified. This may be the result of mutual pressure, so that the cells become polygonal (e.g. in the liver) or it may be the result of active growth and differentiation which produces elongated forms or cells with irregular outlines (e.g. many neurons). During life cells may change their shape. This is well shown by the amoeboid movements of leucocytes when they are examined on a warm stage microscope whilst they are still alive.

The size of an individual depends on the number of its cells rather than on their size: small animals do not necessarily have small cells. The cells of the human body vary greatly in size: the average tissue cell is between 10 μm.* and 20 μm. in diameter, while the small lymphocytes are about 6·5 μm., the large nerve cells about 120–160 μm., and the ovum may have a diameter of 250 μm.

Every cell consists of *protoplasm* surrounded by a cell-membrane: the main mass of the protoplasm forms the *cytoplasm*, and in this is embedded one, or occasionally more than one, *nucleus*. The nucleus is of such vital importance to the cell that if it is lost the "cell" cannot live long and cannot divide (e.g. erythrocytes). Living cytoplasm according to modern researches appears to be structureless at the microscopic, but highly organized at the ultramicroscopic, level. It is, for the most part, fluid or semi-fluid and within it are contained bodies of varied refractive index. Where the refractive index of an inclusion (e.g. a mitochondrion) is very close to that of the surrounding cytoplasm it remains practically invisible in the ordinary optical microscope, whereas bodies of substantially different refractive index (e.g. the nucleus, secretion granules, etc.) will be visible to different degrees. The former structures, however, will often be clearly visible when the living cells are examined by phase contrast microscopy. The constitution of the cytoplasm will vary in relation to the age and metabolic state of a cell and different kinds of cells will show considerable and characteristic variations (e.g. muscle cells, erythrocytes, fibroblasts, and nerve cells).

Cytoplasm cannot at present be defined in strict chemical terms. It consists of an aqueous phase containing various inorganic ions, together with small organic molecules (e.g. carbohydrates, amino-acids) and macromolecules such as polypeptides, proteins and nucleic acids. These are themselves submicroscopic but they are often organized into microscopic organelles with clearly defined morphology and functions. Physically and chemically the cytoplasm is in a state of flux and there is much evidence to suggest that there is actual movement of water and ions and possibly membranes within the cytoplasm. The static picture of the cell so readily obtained from the microscopic examination of fixed tissue is extremely misleading.

A. The Surface Membrane of the Cell (Plasma Membrane)

The cell is covered by a membrane of extreme thinness, which has considerable elastic properties and is capable of repair after local damage. One theory suggests that it consists of a lipo-protein complex, where a bimolecular layer of lipid is sandwiched in between an outer and an inner layer of protein molecules. The lipid is a phospholipid, whose polar (water miscible) groups are held in an outer and inner aqueous phase. The protein molecules are

* 1 μm. = one micrometre or one-thousandth part of a millimetre.
1 nm. = one nanometre or one-thousandth part of a micrometre.

associated with these aqueous phases and their molecular axes are probably at right angles to the hydrocarbon chains of the phospholipid component. The E/M supports this supposition, since high resolution electron micrographs of plasma membranes show a triple arrangement or *unit membrane* of a pale line lying between inner and outer dark lines. The former is considered to be the lipid; the latter the protein. Other, more recent, theories suggest that the primary arrangement of the phospholipids in the membrane is in the form of spherical micelles. At the present time it is not possible to say which, if either, view is correct.

In some cells the plasma membrane may project from the surface in the form of numerous finger-like extensions known as microvilli. The individual villi are not separately resolvable by

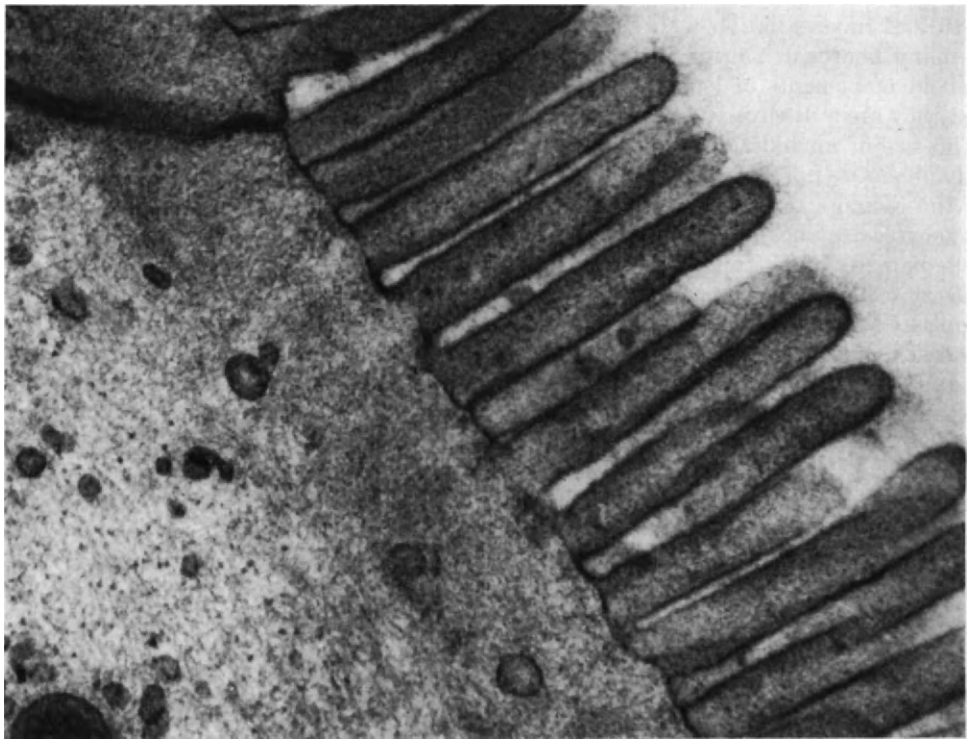

FIG. 2.1. An electron micrograph of the microvilli on the luminal border of an intestinal epithelial cell. These microvilli, which serve to increase the absorptive surface area of the cell, are coated with a filamentous "glycocalyx"; notice the area of the cytoplasm just beneath them is free from organelles and is occupied by the microtubules of the so-called terminal web which may be involved in the maintenance of the form of the microvilli. 0·1 μm.

the light microscope, but in mass they constitute the so-called "brush" or "striated" borders of intestinal epithelial cells and the cells of the proximal convoluted tubules of the kidney. Microvilli appear to be a device for increasing the cell surface area (see Fig. 2.1). In thyroid follicle cells (Fig. 15.7A) and certain others, the length and frequency of microvilli appear to vary with the activity of the cell.

In many cells the plasma membrane has been shown by the E/M to possess a fibrillar, somewhat electron-dense external coating (Fig. 2.2). Histochemical studies have shown that this coating may be considered to be a mucoprotein; Bennett has called this outer layer the "glycocalyx". Although it is certainly of widespread occurrence it is not yet possible to say with certainty that it is a universal feature of cells. Evidence from improved E/M techniques has

shown that in certain situations, e.g. on the microvilli of the intestinal epithelial cell and on microvilli of the syncitiotrophoblast, the glycocalyx is filamentous, with the filaments arranged at right angles to the outer layer of the unit membrane of the cell with which they appear to fuse. On these grounds some authors would regard the glycocalyx as being more properly regarded as an integral part of the cell membrane rather than as an external coat of the cell. Whatever its status, the glycocalyx certainly plays an important role in the life of the cell. It has been suggested that the glycocalyx on free cell surfaces acts to control cell permeability and, because of its highly specific composition, to control cell immunity. If the glycocalyx varies in composition from cell to cell or from one part of the cell to another, some function could also be envisaged in the control of cell to cell interactions which probably form a vital part of morphogenesis and the maintenance of tissue organization. When the glycocalyx on

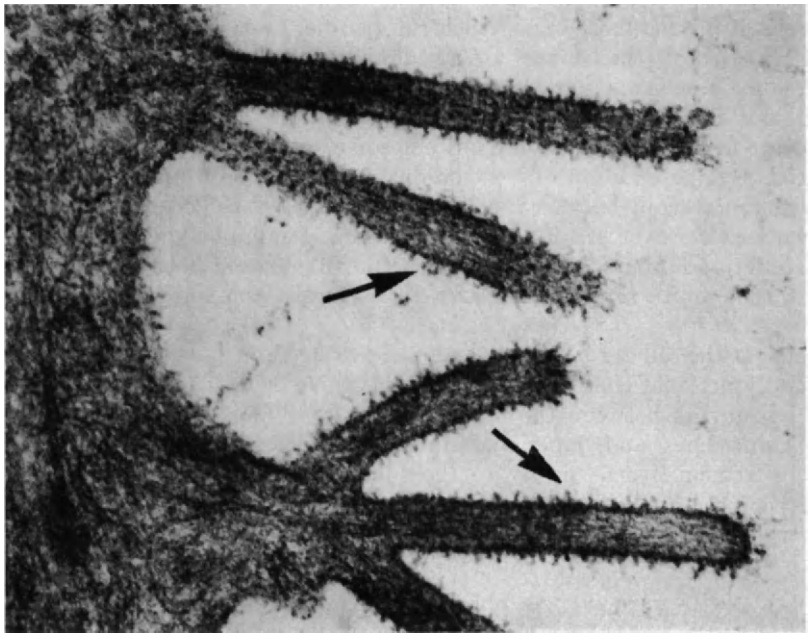

FIG. 2.2. An electron micrograph of microvilli on the surface of human trophoblast. The glycocalyx is apparent (arrows) as filaments extending outwards at right angles to the plasma membrane of the cell surface. 0·1 μm.

the free borders of intestinal epithelial cells is examined, some evidence suggests that enzymes might be located in this region of the cell in order to carry out the terminal hydrolytic digestion of carbohydrates and proteins. The voluminous literature on surface coatings of animal cells and their composition and function is well reviewed by Rambourg (1971).

The E/M reveals that the absorptive epithelial cells of small intestine and kidney have plasma membranes at their basal surfaces which may become folded into the underlying cytoplasm. These form membrane-lined pits or tubules which appear subsequently to become detached from the surface to form membrane-bounded vacuoles in the cytoplasm (Fig. 13.12). Structures such as these are considered to provide evidence for the occurrence of the process known as *pinocytosis*, whereby liquid or macromolecules are taken into cells from their external environment. This cellular activity, well known as occurring in protozoa and certain cells in tissue culture, seems likely to be more widespread than has hitherto been suspected. Since the fluid ingested in this way will nearly always contain substances in solution, pinocytosis can,

in principle, facilitate the entry of essential molecules into those cells which make use of this mechanism. There is evidence, for example, that small fat droplets may enter into intestinal epithelial cells in this way, although it is doubted whether this is the only (or, indeed, the most important) way of entry of lipid.

In all cells, the plasma membrane acts as a barrier between the cytoplasm and the external fluid environment, preventing the two from mixing. It also controls through its capacity of variable and selective permeability the physical exchanges manifested in diffusion and osmosis.

In situations, where cells are grouped together into sheets (as in epithelia) there may be considerable interdigitation between the plasma membranes of adjacent cells. This clearly increases the surface of contact as well as subserving a mechanical function.

With the E/M, differentiated areas can often be seen in the plasma membrane between adjacent cells. These areas are known as *desmosomes* (Fig. 3.6) or *maculae adhaerens*; they consist of areas of plasma membrane which run parallel to each other at a distance of about 25 nm. and which have thickenings or accumulations of electron-dense material on their cytoplasmic side. Often there is an electron-dense line in the intercellular space, although some would regard this line as an artifact due to diffraction in the E/M. Microtubules are commonly seen to converge in the cytoplasm onto a desmosome and they may terminate in the electron dense material. By the addition of electron-opaque marker substances to the fixative fluids used in electron microscopy another type of specialized cell to cell contact has been recognized. This is known as a *nexus* or *gap junction*; the plasma membranes run parallel to one another in close proximity and often a globular substructure is apparent between the cell membranes. The nexus is thought to represent an area for electrical communication between adjacent cells.

In epithelia, at the cellular boundaries adjacent to the lumen, a more complex arrangement known as the "junctional complex" exists; this is thought to act as a seal or barrier to the diffusion of substances between the cells in the intercellular matrix (see later, p. 40 and Fig. 3.7). Desmosomes and junctional complexes are regarded by most workers as areas specialized for attaching cells firmly to their neighbours, but recent thinking has tended towards the view that they, together with the nexus, are important as a means for ensuring rapid conduction of excitation from cell to cell.

B. Cytoplasmic Contents

The ground substance of the cytoplasm, in so far as it can be adequately described at all, consists of a watery solution of salts, proteins, carbohydrates, together with ribonucleic acid and lipids. Cytoplasm has colloidal properties and can fluctuate between the sol and gel condition; the peripheral zone of the cytoplasm of many, if not all, cells appears to be permanently gelated. Some of the chemical constituents of cytoplasm, the proteins and lipids in particular, appear to be organized in the form of a "cytoskeleton" consisting of membranes and microtubules. This "cytoskeleton", however, must be regarded as a labile rather than a rigid structure in the mechanical sense and must be so constituted as to permit the streaming movements of the cytoplasm which are known to occur, for example, in living fibroblasts, in the tips of growing nerve fibres and perhaps in all cells. The microscopically visible structures in cytoplasm of living and of fixed cells may be divided into two groups, with perhaps some overlapping between them: (1) *Organelles:* these are specialized parts of the living substance, often able to divide, and probably always present. (This group includes mitochondria, Golgi apparatus, membranes, centrosphere, lysosomes and microtubules.) (2) *Inclusions:* these are very commonly the products of cellular activity and are often of a temporary nature. (This group includes fat droplets, glycogen particles, pigment granules, crystals and secretion granules.)

(1) Organelles

(*a*) **Mitochondria** (chondriosomes). Most cells contain mitochondria, either as granules varying from 0·5 μm. to 2 μm. in diameter, or as filaments which may be up to 5 μm. in length. (Fig. 1.3). Their number varies according to the physiological state of the cell, and they can be demonstrated by supravital staining with Janus green B or tetrazolium salts which appear to stain them specifically because of their content of oxidative enzymes. The investigation of ultrathin sections of these bodies by electron microscopy shows that they are bounded by a double membrane, from the inner of which a series of internal folds (cristae mitochondriales) extend into the mitochondrial matrix (Fig. 2.3). Mitochondria have also been isolated in bulk,

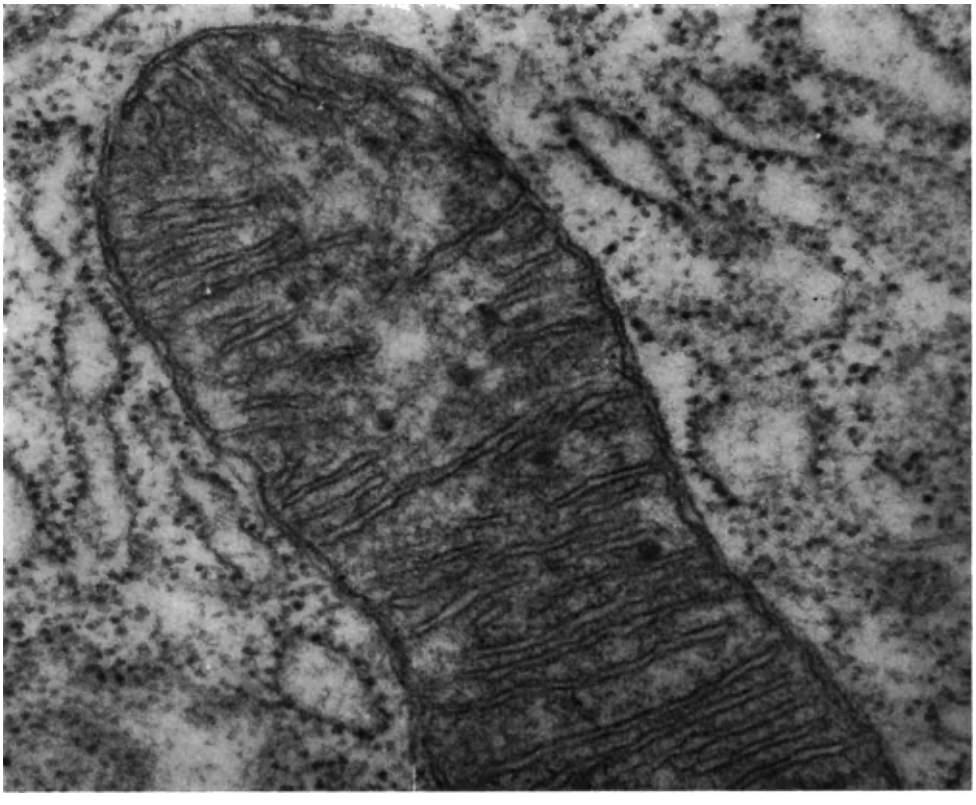

FIG. 2.3. An electron micrograph of a mitochondrion in a pancreatic exocrine cell. Notice the cristae projecting into the matrix of the organelle. 0·1 μm.

particularly from homogenized liver preparations, and they have been shown to contain phospholipid, protein, small amounts of nucleic acids and a variety of respiratory and other enzyme systems. Recent experiments involving the fragmentation of mitochondria by ultrasonics suggest that whereas some, at least, of the enzymes of the Krebs cycle are located in the central matrix, those of the electron transport and associated oxidative phosphorylation systems are situated in the membranes, including those of the cristae. The subunits of the membranes (the so-called elementary particles) which have been visualized by negative staining techniques are now thought to contain an enzyme which participates in coupling the phosphorylation of ADP with electron transport. As seen in living cells by dark-ground illumination and by phase-contrast microscopy, the mitochondria appear to show independent

powers of movement. They are also capable of transverse division, and are readily altered by temperature, autolytic and other changes, when they may swell up and finally disappear.

(*b*) **Golgi Apparatus.** This structure, in material suitably treated with osmium tetroxide or silver nitrate, appears with the optical microscope to consist of an anastomosing network of threads of varying thickness, and has been demonstrated in nearly all cells. It is difficult to make out in the living condition, although in certain cells a system of canals has been reported to occupy the Golgi zone. Its position in the cell and its extent vary: in nerve cells the Golgi apparatus is usually perinuclear (Fig. 11.6), while in secreting cells it is polarized, occupying a position between the nucleus and the secretory border of the cell. This part of the cell is often associated with small droplets of lipid (lipochondria), PAS-positive material (probably mucoprotein) and phosphatases, especially the so-called thiamine pyrophosphatase (TPPase).

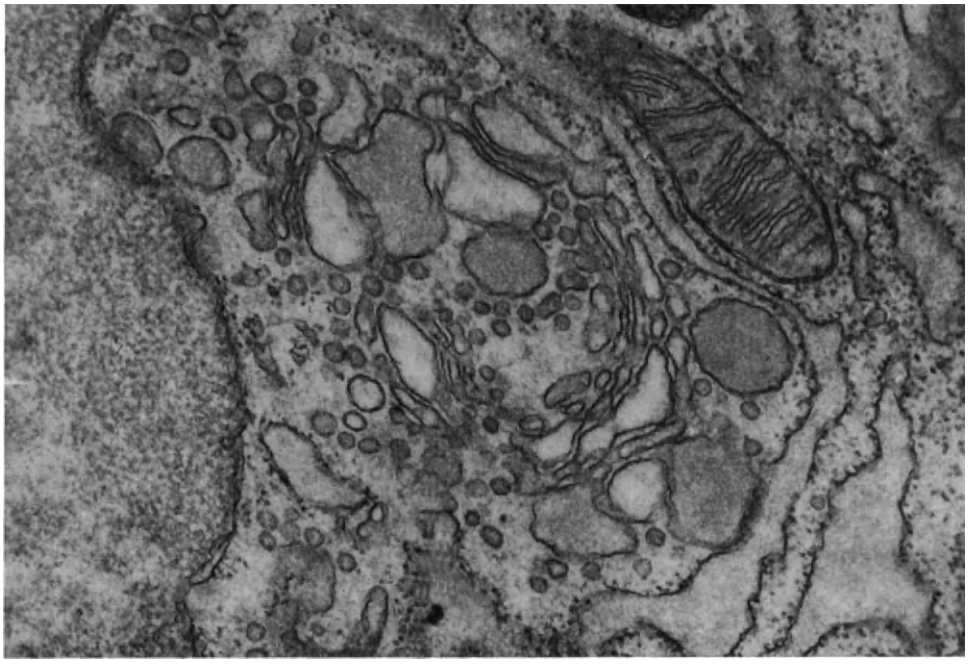

FIG. 2.4. An electron micrograph of the Golgi apparatus in a plasma cell. The typical flattened sacs, small vesicles and the large vacuoles of the Golgi apparatus may be seen. Part of the nucleus of the cell is on the left of the micrograph, and a nuclear pore is present in it at the bottom of the picture. 0·5 μm.

Electron microscopy shows this region of the cell to possess a fairly characteristic ultrastructure consisting of very small membrane-bound vesicles, large membrane-bound vacuoles and groups of paired membranes often arranged in stacks (Fig. 2.4). The black networks which result from impregnation with silver and osmium represent the deposition of these metals upon all or some of the components mentioned above.

In secretory cells, such as those of the pancreas and adenohypophysis, the secretion granules are often first detectable in the Golgi area. Studies with the E/M show that secretion granules appear in the membrane-bound vacuoles. There is little doubt, therefore, that this region of the cytoplasm is intimately involved in the secretion process, and probably is the site where the secretory materials are condensed into granular form. Recent work with E/M autoradiography has confirmed this view and has shown, for example, that it is in this region that the sulphated mucoprotein of the intestinal epithelial goblet cell is elaborated Its

function in non-glandular cells is still a matter for speculation—it might be a centre for the synthesis of new membranes.

(*c*) **Centrosphere.** Almost every cell contains near the nucleus a small area of condensed cytoplasm in which are found two minute dots, the centrioles. These bodies are concerned with mitosis and will be described more fully below. The centrosphere of large multinucleated cells may be large, with several groups of centrioles: it is said to be absent from highly differentiated cells that have lost the power of division, such as nerve cells.

(*d*) **The Endoplasmic Reticulum** (Ergastoplasm, chromidial substance). Garnier was the first to point out, many years ago, that the basal region of the cytoplasm of many kinds of glandular cell often stained differently from the rest. These special regions often had a striated

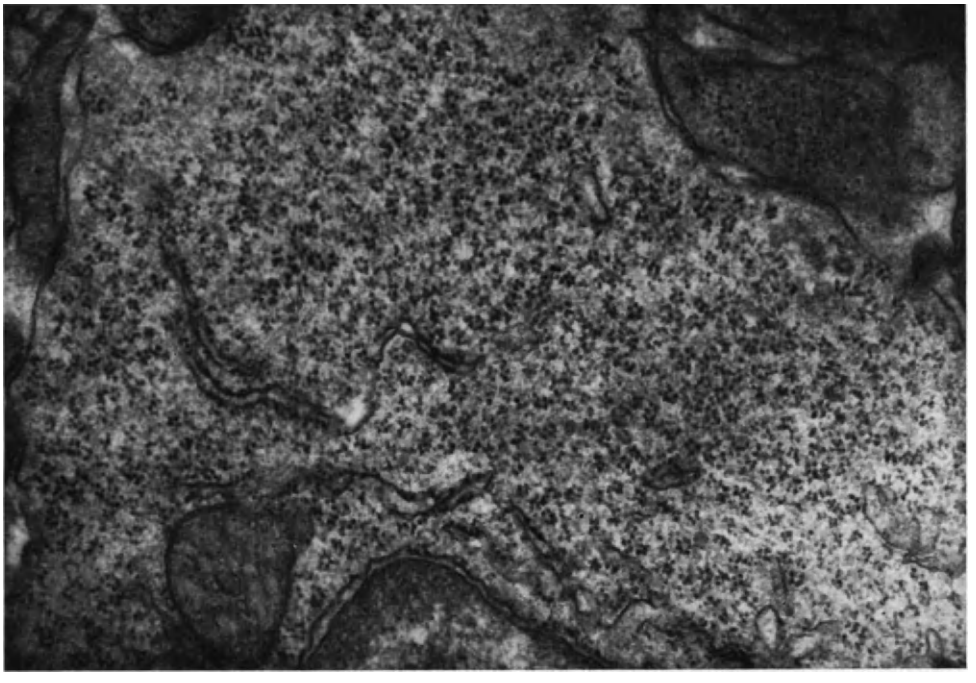

FIG. 2.5. An electron micrograph of free ribosomes (many associated into the rosettes and strands of polyribosomes) in the cytoplasm of a lymphoblast. One or two fragments of membranous endoplasmic reticulum are also visible. 0·5 μm.

or streaky appearance, and because Garnier's observations led him to the conclusion that these areas differed chemically from the rest of the cytoplasm and might be concerned in some way with the secretory process, he designated them *ergastoplasm*. More recently, it has been established that the ergastoplasm, like the nucleoli of the nucleus, is particularly rich in ribonucleic acid (RNA). Since RNA is both polymerized and acidic it has a strong affinity for many basic dyes such as pyronin, methylene blue and toluidine blue; it is markedly *basiphil*. This affinity is destroyed when the RNA molecule is hydrolysed by the enzyme ribonuclease, for which it acts as a specific substrate. Cytoplasmic basiphilia is by no means confined to glandular cells. Actively growing cells and any cell intensively engaged in the synthesis of protein are likely to contain cytoplasmic RNA although in these cases the RNA-containing particles (the *ribosomes*) are not associated with any membranes but occur free in the cytoplasm (Fig. 2.5).

One of the most important discoveries in cell biology of recent years has been the demon-

stration that RNA is a molecule directly concerned in determining the order in which amino-acids are condensed together to form specific protein molecules. As will be briefly discussed later, it is the DNA of the chromosomes which predetermines the activities of cytoplasmic RNA, so that there is a chemical link between the nucleus and protein synthesis in the cytoplasm. Differential centrifugation, together with radioactive isotope techniques and electron microscopy have demonstrated conclusively that the ribosomes contain RNA and that they represent the sites of cytoplasmic synthesis of proteins from amino acids.

Electron microscopy of the ergastoplasm shows that it may possess two kinds of ultrastructural organization. The first and most complex is that seen in glandular cells, such as pancreatic exocrine cells which are secreting protein "for export". Here it consists of an elaborate array of intracellular membranes which form flattened sacs or "cisternae", the outer surfaces of which are studded with the ribosomes (Fig. 2.6). The cisternae are presumably fluid-filled in the living cell. The whole complex of membranes and ribosomes together form what is known as the *granular* or *rough-surfaced endoplasmic reticulum*. The second category of endoplasmic reticulum is the *smooth* or *agranular* form, where the membranes form an anastomotic three-dimensional network of tubules (Fig. 2.7). Ribosomes are absent and in consequence cytoplasm which is rich in agranular endoplasmic reticulum is often acidophilic in its staining reactions. Agranular endoplasmic reticulum may occur in the same cells as the granular form and indeed is often continuous with the latter, as in the liver cell; other cells such as the interstitial cell of the testis and the cortical cells of the adrenal contain only the agranular reticulum. Detailed knowledge of the function of agranular endoplasmic reticulum is not yet available but present evidence indicates that it is involved in steroid synthesis, in lipid metabolism and transport, and in detoxification processes.

(*e*) **Lysosomes.** These are membrane-bound organelles of the same order of size as mitochondria. They are limited by a single unit membrane and have a variable electron density. Often they are seen to contain "myelin figures", i.e. whorl-like aggregates of membranes derived from the lysosomal breakdown of cellular constituents. Lysosomes contain various hydrolytic enzymes, including cathepsin, proteases, glycosidases and acid phosphatase and are concerned in intra-cellular digestive processes and in the breakdown of material ingested by pinocytosis. Lysosomes occur in a wide variety of cell types, especially in macrophages and other cells of the reticulo-endothelial system (see Fig. 6.7).

The microbodies, found in liver and kidney tubule cells of mammals, are possibly related to lysosomes. Microbodies have a variable morphology but in liver cells they often contain electron-dense "cores" which appear to be composed of numerous regularly arranged tubules. The enzymes urate oxidase, d-amino acid oxidase and catalase have been demonstrated to occur in the microbodies. As these enzymes are capable of reducing oxygen to hydrogen peroxide and this latter compound to water, it has been suggested that a better name for the microbodies would be *peroxisomes*. The precise role of these organelles in the cell is not yet understood, but it has been suggested that they are important in the process of gluconeogenesis.

(*f*) **Microtubules.** With the introduction of aldehydes as fixatives for the E/M, the presence of tubules in the cytoplasm became apparent. These are the microtubules; they are long, often running for distances of several micrometres in any one section and are about 20–30 nm. in diameter with walls 5 nm. thick believed to be composed of globular sub-units.

The microtubules often converge on the centrosome with its centrioles and in cell division they appear in the cytoplasm in great numbers to form the spindle fibres. Microtubules are often found in conditions where cellular stiffening is required, where they act as a cytoskeleton. Examples of this are the microtubules in the core of the microvilli of intestinal epithelial cells and around the margin of the oval nucleated erythrocytes of amphibians. In addition to a cytoskeletal function it has also been suggested that orientated groups of microtubules may play an important role in defining channels of rapid intra-cellular transport.

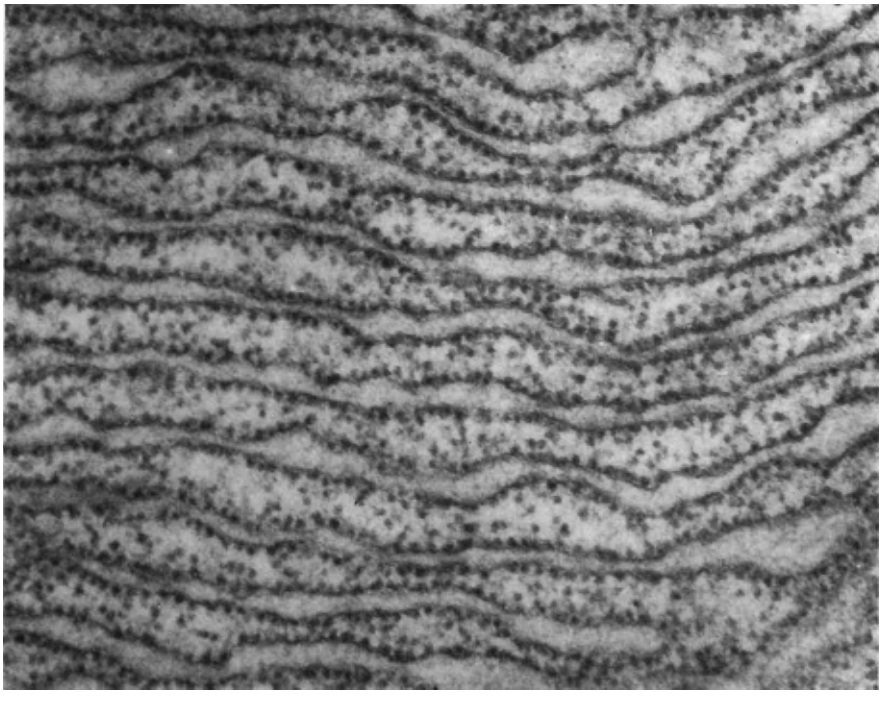

FIG. 2.6. Rough-surfaced endoplasmic reticulum in the cytoplasm of an exocrine pancreas cell. The outer surfaces of the membranes are seen to be covered with the ribosomes.

0·1 μm.

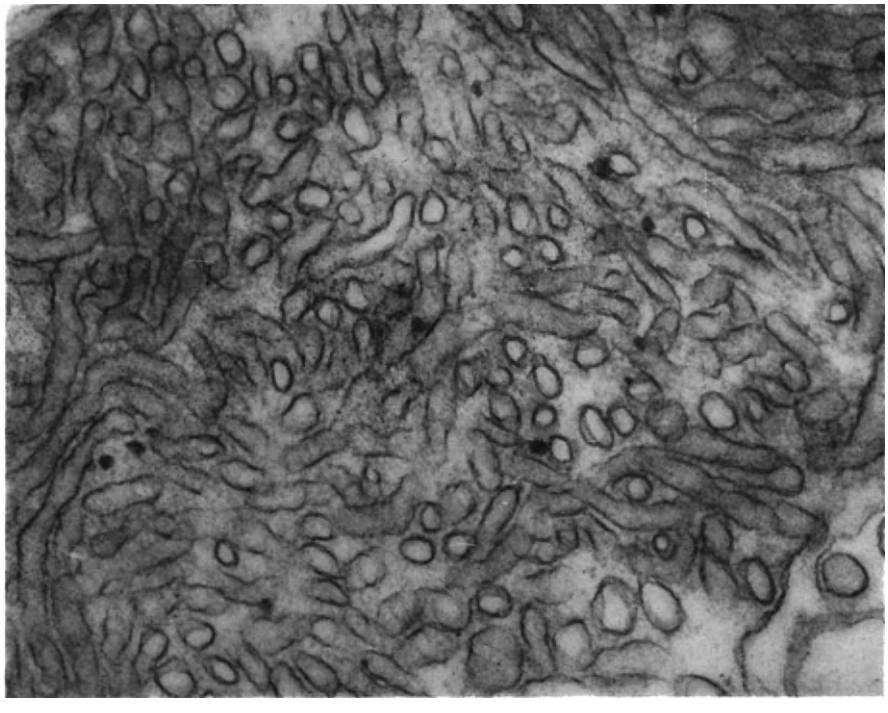

FIG. 2.7. Agranular endoplasmic reticulum in the cytoplasm of a cell from the adrenal cortex. Notice that this type of ER is composed of anastomosing tubules and that ribosomes are absent.

0·1 μm.

(2) **Inclusions**

(*a*) **Lipid Droplets.** Special fixation and subsequent treatment are necessary for the demonstration of lipid droplets, because in the preparation of the usual stained section these have been dissolved out and are merely represented as holes. The amount of lipid material varies greatly and much of it is in the "masked" form, i.e. it is in combination with other substances and not histologically demonstrable. The amount of stainable fat in the cells may be enormously increased in certain diseases. Such fatty deposition often occurs in liver and heart cells following cellular hypoxia, in diabetes and in liver cells following poisoning by carbon tetrachloride or ethyl alcohol.

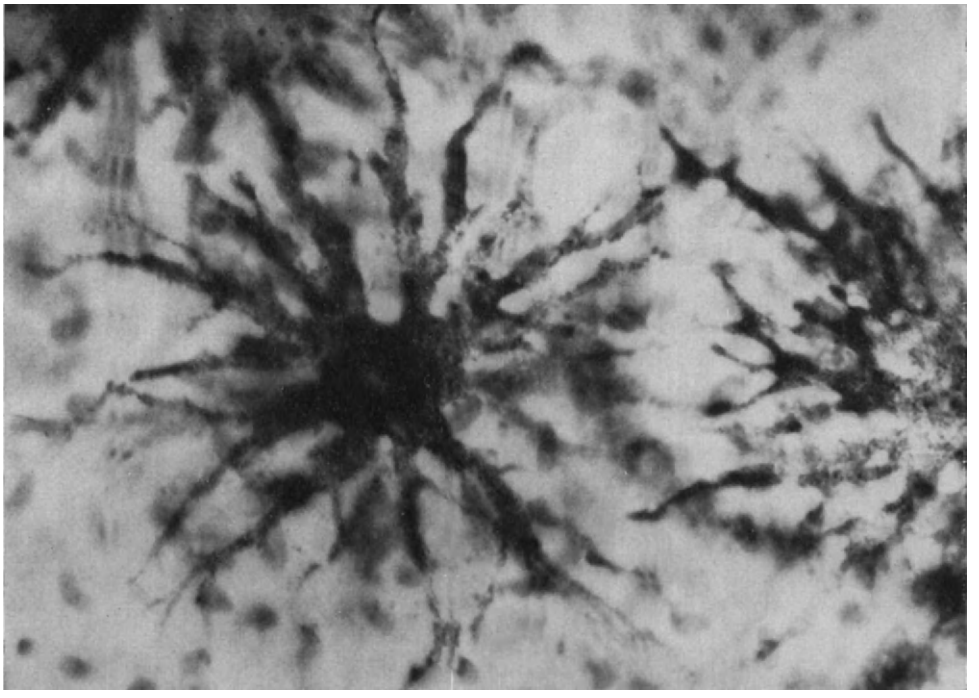

FIG. 2.8. Melanocytes, with typical branching form, in the epidermis of an amphibian. 10 μm.

(*b*) **Carbohydrate.** Glycogen is dissolved by aqueous fixatives, but it can be demonstrated by fixing the tissue in absolute alcohol, or in fixatives containing picric acid.

The histochemical demonstration of glycogen in tissue prepared by the freeze-drying technique suggests that it is really present in an extremely finely divided form—the somewhat coarse droplets, seen after other methods of fixation, being the results of coalescence. The amount present varies with physiological conditions, but it is commonly found in liver cells, cartilage cells, muscle cells, leucocytes and the superficial squames of the vaginal epithelium.

(*c*) **Pigment.** Many cells throughout the body contain pigment granules of various kinds. The breakdown products of haemoglobin can be regarded as foreign matter: the pigments formed by the cells themselves (autogenous) are of three kinds, namely, melanin, lutein and lipochrome, sometimes called lipofuscin.

(i) *Melanin.* Pigments of this group are widely distributed in the body, being found in such diverse situations as in the choroid coat and iris of the eye, in the hairs, in the Malpighian layer of the epidermis, although in this particular site the melanin is not normally autogenous (see

Chapter 17), in the pia mater, and in the substantia nigra cells of the brain. (In Amphibia the pigment, occurring in branched melanophore cells (Fig. 2.8), can condense or disperse: this is used as the basis of the melanophore reaction for pituitary hormones.) Under abnormal conditions the pigment may increase in amount and become deposited in the epithelial cells of the skin (e.g. pigmented naevi, and bronzed skin of Addison's disease). Albinos lack the melanin group of pigments.

(ii) *Lutein.* This is the yellow, soluble colouring matter of luteal cells and fat tissue.

(iii) *Lipochrome* (lipofuscin). Particularly in the later years of life a fine, brown-yellow pigment of a lipid nature appears in increasing amounts in cells of the cerebral cortex, in certain ganglion cells (particularly of the autonomic system), in liver cells, in cells of the adrenal cortex, seminal vesicles and testis, and in heart muscle cells: in these last it collects at the poles of the nuclei. These lipid pigments are often referred to as "wear and tear" pigments. From E/M and histochemical studies the concept is developing that lipid pigment granules represent deposits of insoluble cell waste products or organelles which have been partly digested by the lysosomes. If the increase of pigment is associated with wasting of the part the condition is spoken of by the pathologists as "brown atrophy".

(*d*) **Crystals.** Cells sometimes contain crystals. They have been described in the interstitial cells of the testis (crystals of Reinke), in liver cells where they appear in the cisternae of the granular endoplasmic reticulum, and in yolk platelets of amphibian oocytes. Crystals in cells are usually assumed to be protein but this has only been shown conclusively in a few cases: in the majority of cells the nature and function of any crystalline deposits are not known.

(*e*) **Secretory Granules.** These characterize many kinds of gland cells and are the visible immediate precursors of their specific secretions. They are generally proteins or polypeptides and are synthesized in close association with the endoplasmic reticulum where they generally first appear in the cisternae, subsequently moving up into the Golgi area. Secretion granules can often be seen in living cells by phase contrast microscopy and are readily stained in sections of suitably fixed material.

C. The Nucleus

This is usually a spherical structure, but it shows variations in shape in accordance with the shape of the cell: thus it is elongated in smooth muscle, flattened in squamous epithelium, and ovoid in columnar cells. It also varies in position in accordance with the bulk of cytoplasmic inclusions, being pushed to the periphery in adipose cells or active gland cells, and remaining in the middle of the resting gland cell: occasionally it is lobulated, as in polymorphs and in marrow megakaryocytes. Usually each cell contains only one nucleus, but some cells may contain two (e.g. liver, bladder epithelium, myocardial fibres). It is marked off from the surrounding cytoplasm by the nuclear envelope. This disappears during the prophase of nuclear division. The nucleus contains at least one (and often several) nucleoli (Fig. 2.9). These are normally spherical bodies, rich in RNA which vanish during mitotic prophase. In growing cells, glandular cells and cells otherwise engaged in active protein synthesis, the nucleoli commonly become enlarged, suggesting that they or, more probably, their RNA is directly concerned in this process.

Recent investigations with the E/M show that probably in all cells the nuclear envelope (1) consists of *two* membranes separated by a space (Fig. 2.4) and (2) possess pores. It is conceivable that the latter provide a pathway whereby nuclear RNA (such as that mentioned above) gains access to the cytoplasm.

In addition, in fixed and stained cells, there are smaller intranuclear granules which colour strongly with basic dyes. These are rich in DNA and represent the only readily stainable parts of the chromosomes in the interphase nucleus. During the prophase of cell division (see below) regions of the thread-like *chromosomes*, hitherto unstained, acquire an increasing affinity for dyes and, in consequence, at this stage the whole chromosome, with the exception

of the spindle attachment region, becomes deeply stainable with basic dyes. The basic protein of the chromosome (histone) and its associated nucleic acid is known as *chromatin*. The genes, associated with the mechanism of inheritance, are distributed in a linear fashion along the chromosomes (see below).

The chromatin and the nucleolus are dispersed in what was called by the classical cytologists the "*nuclear sap*". Recent E/M studies have shown that the material in between the interphase chromosomes consists of numerous granules of varying size. It has been suggested, therefore, that the term "*nuclear matrix*" would be more appropriate.

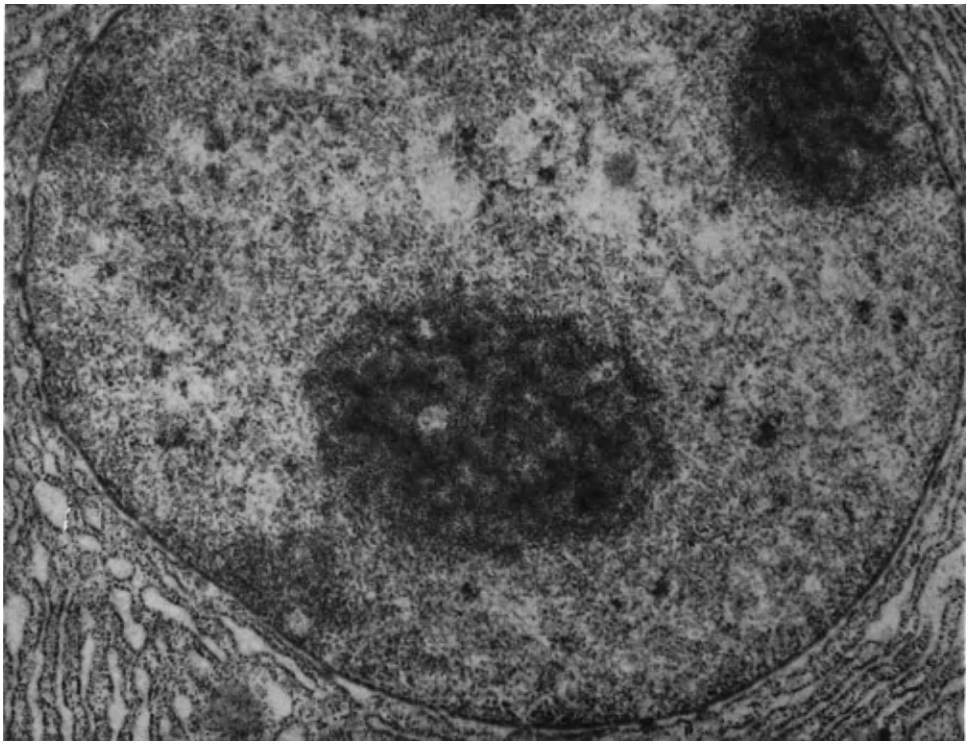

FIG. 2.9. An electron micrograph of the nucleus of a pancreatic exocrine cell. Two prominent nucleoli are visible. 1 μm.

The E/M has yielded surprisingly little information on the detailed submicroscopic morphology of the chromosomes. This is largely due to the facts that:

(i) osmium tetroxide, normally used to fix the material, does not impart much electron density to the chromatin;
(ii) the chromosomes are not sharply delimited by membranes as are the cytoplasmic organelles;
(iii) E/M sections are so thin that only a very little of the total length of each chromosome will appear in any one section.

The use of aldehyde fixatives has overcome the first of these difficulties and has shown the chromosomes to be composed of what appear to be numerous granules and fine filaments. Recent work has shown that much more information may be obtained by the use of isolation techniques. Chromosomal material is isolated from the nucleus, spread on a carbon-coated E/M grid and stained with heavy metal atoms; under these conditions the central filamentous

"core" is easily studied. The interpretation of such images is not easy and general agreement does not yet seem to have been reached on their significance.

Similarly the nucleolus has been shown to possess a granular ultrastructure (Fig. 2.9). Often a rather dense strand—the *nucleolonema*—is present, embedded in a fibrous or amorphous matrix or *pars amorpha*.

CELL ACTIVITY

Every cell is in a state of continuous activity, capable of carrying out those complicated physical and chemical reactions that are necessary for respiration, synthesis, excretion and other vital activities. The great speed at which chemical processes occur is due to the presence of intracellular enzymes. In addition, in the highly differentiated, multicellular organisms, such as the mammal, special groups of cells are more particularly concerned with special functions for the benefit of the whole organism: these groups of cells deal with processes affecting the metabolism, reproduction and the specialized activities of life. The nucleus is of the greatest importance in these activities: it is itself the seat of many enzymic reactions and is essential for the elaboration of synthetic products.

Respiration. All animal cells take up oxygen from the blood or tissue fluids for use in their metabolic activities: these produce primarily energy, together with carbon dioxide and water which are returned to the blood. This internal respiration is probably carried out by means of intracellular oxidizing enzymes in the presence of cytochrome.

Synthesis. All animal cells take up food materials from the blood or tissue fluids, and make use of them by virtue of their intracellular enzymes. In this way growth occurs by internal reorganization and new compounds are formed by the body.

Excretion and Secretion. All animal cells get rid of the waste products resulting from their own activity by passing them into the blood or tissue fluids. In addition, certain groups of cells are specially modified for removing waste products from the body (kidney epithelium, skin, lungs, liver). Some cells are specialized for the formation of secretions, and in this case secretory granules may be found in them.

Phagocytosis and Amoeboid Movement. Certain cells have the power of engulfing and often destroying foreign bodies, such as débris of dead cells, and bacteria: this is often accomplished by a flow of the cell protoplasm round the foreign body, the process being associated with amoeboid movement on the part of the cells. This property of phagocytosis is shown by the blood leucocytes and all the histiocytic cells scattered throughout the body.

THE CHROMOSOMES AND CELL DIVISION

All cells can undergo division, with the possible exception of such highly differentiated cells as nerve cells, erythrocytes and polymorphonuclear leucocytes.

Direct division (*amitosis*) is said to occur occasionally; in this case the nucleus and the cytoplasm undergo simple constriction with the formation of two nucleated daughter cells.

Much more frequently division is by the complex indirect method of *mitosis*.

The Interphase Nucleus

The principal morphological characteristics of the interphase nucleus have already been described. Recent investigations using cytophotometric and autoradiographic techniques (see Chapter 1) have demonstrated that during the interphase of a nucleus which is subsequently to divide, there is a *replication or doubling* of the DNA. If, for example, the cells of a tissue showing active mitosis are exposed to thymidine containing the isotope of hydrogen called tritium (^3H), the tritiated thymidine is rapidly incorporated into a certain proportion of the interphase nuclei where its presence can be demonstrated by autoradiography. These are

the nuclei which, being in the process of DNA replication at the time, have used some of the labelled thymidine and incorporated it in newly synthesized DNA molecules.

From such studies the whole mitotic cycle has been divided into several phases designated by the letters G_1, S,G_2, and M. G_1 represents the period from the end of the previous division to the start of DNA synthesis which occupies the S phase. G_2 is the second gap period from the end of synthesis to the onset of the actual M or mitotic division phase.

The DNA molecule has certain unique properties; it is long, double stranded, helical (i.e. coiled like a spring) and of high molecular weight. Each strand consists of a chain of phosphoric acid and deoxyribose groups alternately arranged. Each sugar has attached to it a

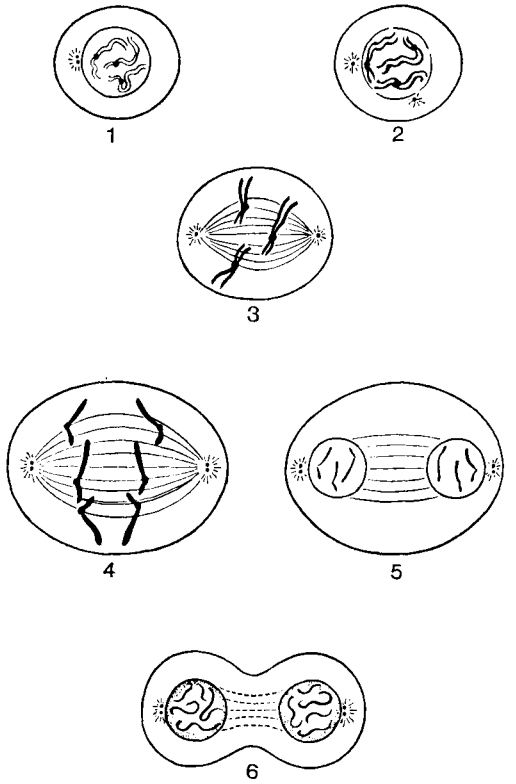

FIG. 2.10. A diagram of the stages of mitosis (1) early prophase (2) late prophase (3) metaphase (4) anaphase (5) telophase (6) division of the cytoplasm.

purine (adenine or guanine) or pyrimidine (cytosine or thymine) base and these are always arranged such that an adenine of one strand is always paired with a thymine of the other and likewise guanine is paired with cytosine. These base pairs are held together by hydrogen bonds. At replication it is presumed that the helical molecule uncoils and the two strands separate. Each strand then acts as a template for the synthesis of its complement and in this way *two* identical stranded DNA molecules are formed from the pre-existing single one. This is the crucial event that occurs during interphase and is the key to the whole mitotic process, which has been arbitrarily divided into stages, as follows:

(*a*) **Prophase.** (Figs. 2.10, 2.13). Here the thread-like, stainable chromosomes appear and each is seen to be longitudinally doubled, except at the centromeres, which will later form

regions of attachment for the spindle fibres. The double chromosomes each composed of two daughter chromatids are the morphological expression of the replication of the DNA which occurred in the previous interphase. Although chromosomes are not customarily seen in interphase nuclei, they are presumed to be present in a physical state which makes them both invisible and unstainable. (Perhaps they are enormously attenuated or hydrated.) It is thus reasonable to suppose that the doubling of the DNA is accompanied by a doubling of each chromosome.

As prophase proceeds the nuclear envelope breaks down, the nucleoli disappear and the chromosomes shorten and thicken. The latter is a consequence of the increasing spiralization of the whole chromosomes.

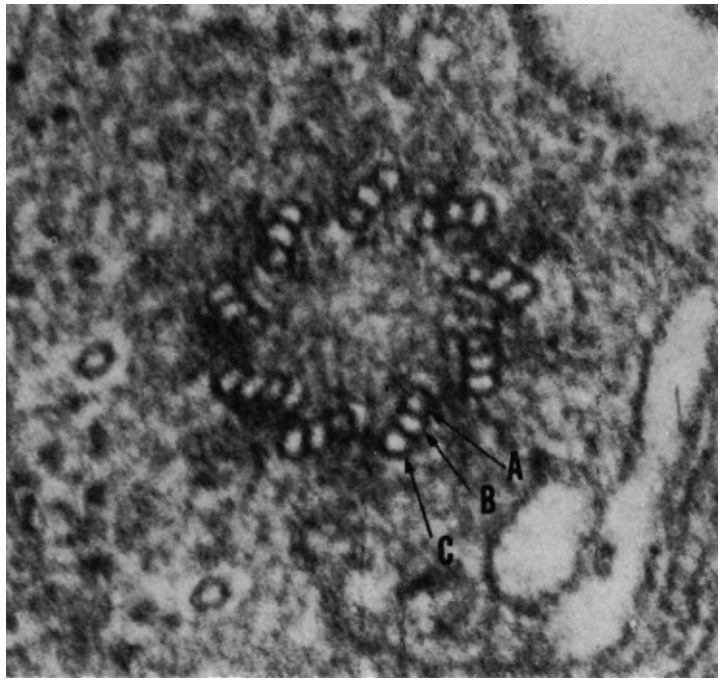

FIG. 2.11. An electron micrograph of a centriole in transverse section. The A, B and C sub-fibres of each group are clearly seen.

While these events are in progress the centrioles begin to move through the cytoplasm and finally take up positions at the opposite poles of the nucleus. Each centriole is a minute cylindrical body, having an ultrastructure closely resembling that of a cilium or flagellum. Electron microscopy has shown that the centriole has the basic structure of a hollow cylinder *c.* 300–500 nm. long, open at one end and closed at the other. In transverse section the centriole has a circular outline with a wall composed of nine groups of what appear to be longitudinally arranged tubules. Each group consists of three sub-units, the innermost being designated the A sub-fibre, the middle B and the outermost the C sub-fibre. The A sub-fibres are equally spaced out around the periphery of a circle about 150 nm. in diameter; each complex of sub-fibres is arranged so that its axis is at an angle of about 30° to the tangent to the circle (see Fig. 2.11). Centrioles usually occur in pairs, each with its long axis perpendicular to that of its partner, so forming what is often called the *diplosome*. In the immediate neighbourhood of the centrioles delicate strands of microtubules progressively form and radiate out in all directions.

Those between the centrioles appear to link up and form a body known as the *spindle*, the others constitute the *aster* (Fig. 2.13).

Prophase is terminated by pro-metaphase when the double chromosomes, as the result of a series of "joggling" movements, align themselves across the centre of the spindle to form an equatorial plate.

(*b*) **Metaphase** (Fig. 2.12). This, in comparison with prophase, is usually of relatively short duration. The centromeres of each chromosome pair split longitudinally and each becomes attached to a spindle fibre. At this stage, therefore, the chromosomes of each pair become

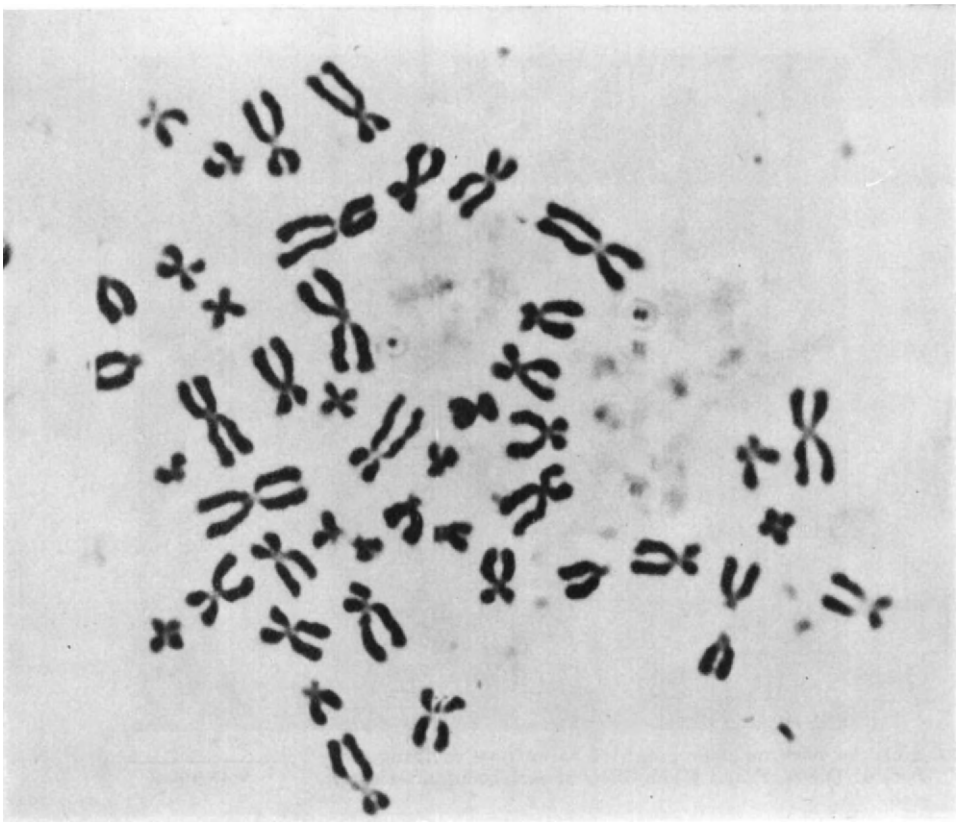

FIG. 2.12. A polar view of a spread out metaphase plate of human chromosomes. Such preparations are valuable for the detection and classification of abnormalities of the chromosomes.

10 μm.

completely separated. It should be noted also, that although some spindle fibres become attached to chromosomes, others appear to pass uninterruptedly from centriole to centriole (Figs. 2.10, 2.13). Recent techniques (such as staining with fluorescent dyes) have greatly assisted in the identification of individual chromosomes in a metaphase preparation. The arrangement, in order, of the chromosomes from photographs of the metaphase stage of cell division, is termed a *karyotype*. Karyotypes are proving more and more useful in medicine with the realization that some clinical conditions are due to chromosomal abnormalities. A typical example is the presence of an extra chromosome number 21 to give Down's syndrome (mongolism).

(*c*) **Anaphase.** This is an immensely active stage in mitosis, in which each member of a

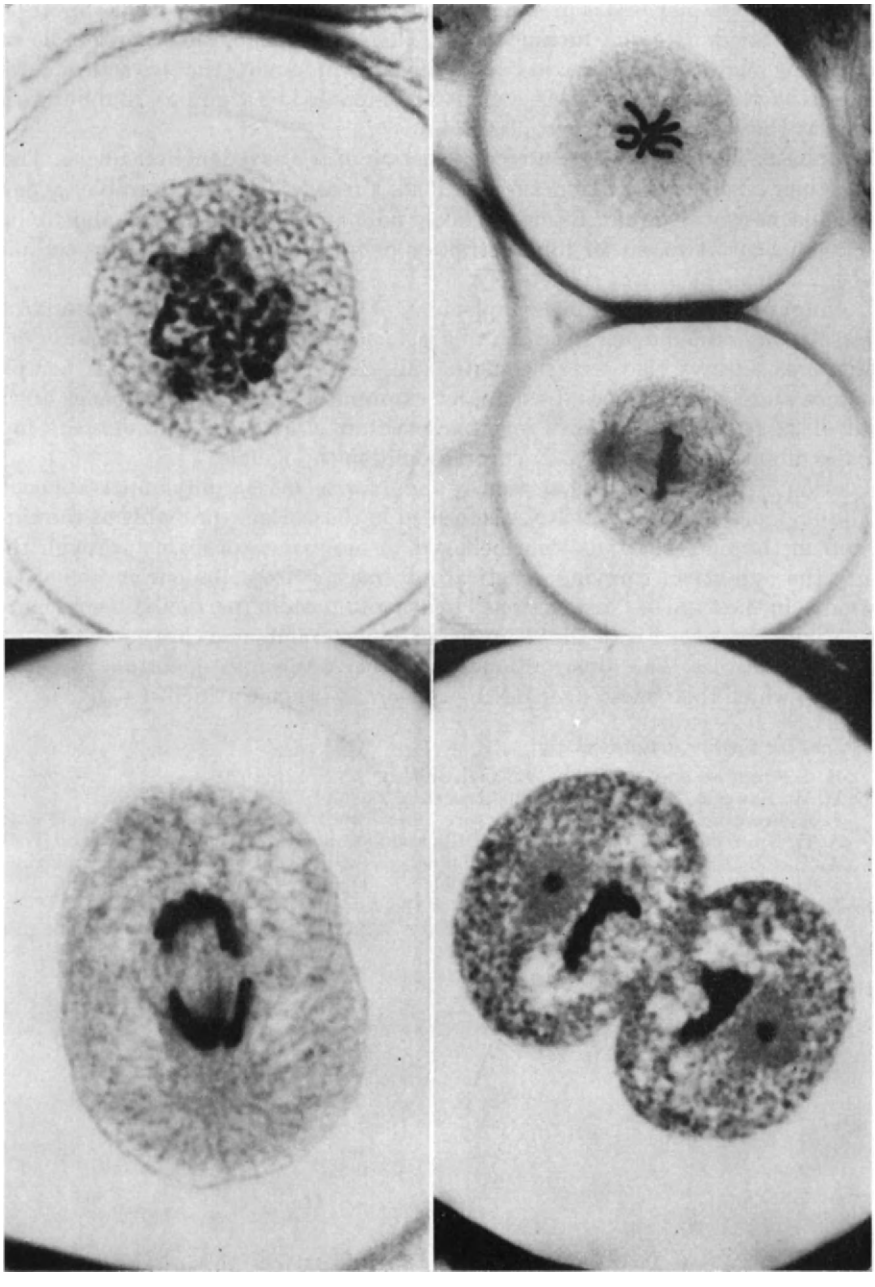

FIG. 2.13. The successive stages of mitosis, photographed in the cleavage divisions of *Ascaris megalocephala*; prophase (top left), metaphase, one cell in polar and one cell in equatorial view (top right), anaphase (bottom left) and telophase (bottom right). Notice the prominent asters and spindle fibres in the metaphase and the centrioles and centrosphere in the telophase stage.

chromosome pair appears to be pulled by its spindle fibre to the opposite pole of the cell (Figs. 2.10, 2.13). Whether this is precisely what happens, rather than what appears to happen, is still a matter for debate and further study. There is evidence that the spindle as a whole elongates during anaphase and this, in itself, will assist in moving the two sets of chromosomes apart. In cells such as fibroblasts, this stage is accompanied by vigorous "bubbling" and other movements at the surface of the cytoplasm.

(*d*) **Telophase.** This is the stage of reconstruction of the two daughter nuclei. The chromosomes lose their visible spiral structure and soon cease to be recognizable, a new nuclear membrane and new nucleoli are formed. The spindle and aster disappear and the cytoplasm becomes divided into two, either by constriction or by the formation of new cell membranes (Figs. 2.10, 2.13).

Each daughter cell receives its complement of mitochondria, Golgi material and other organelles, although the manner in which this is achieved is by no means properly understood.

Mitosis thus ensures that every somatic cell receives its full (diploid) complement of chromosomes and hence of genes. In man, for example, this diploid number is 46 (Fig. 2.12) composed of 22 pairs of autosomes together with an X and a Y chromosome in the male and 22 pairs of autosomes and two X chromosomes in the female.

Replication of DNA and its separation at anaphase is not the only important occurrence in nuclei. The evidence suggests that RNA is formed in the nucleus, probably at the chromosome surface and in the nucleoli. It is then believed to move out (probably through the nuclear pores) into the cytoplasm carrying genetical information from the chromosomal DNA. This information is in the form of "instructions" relating to protein (particularly enzyme) synthesis and is especially concerned with the sequence in which amino-acids are condensed together to form specific molecules. The "instructions" probably come into operation at the ribosomes (see earlier) to which this "messenger RNA" appears to become attached.

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CHAPTER 3

EPITHELIA

The embryonic cell has the potential ability to carry out many and varied kinds of cellular activity with equal ease, but as development proceeds groups of cells become specialized for the carrying out of particular functions; in this way tissues are formed. As differentiation proceeds the cells of a tissue frequently become separated by the formation of intercellular substance often containing non-living products of cell activity, e.g. fibres. The amount of this substance varies greatly in amount in different tissues and is of great importance in the functional activities of that tissue.

The basic tissues are as follows:

(1) Epithelia.
(2) Connective tissues, including cartilage, and bone.
(3) Muscle.
(4) Nerve.

Blood and lymph are often included as connective tissues.

Examination of thin slices of stained tissues, as is customary in class work, often gives a very false impression of the true nature of the tissue as a whole. It is true that a large amount of detail is seen in individual cells but this is only a two-dimensional representation of what is really a solid organ, possessing three dimensions. Many of the difficulties of the student beginning histology arise from a failure to appreciate this fact; constant practice is needed to translate the appearance of sections back into three dimensions and so add the missing information.

Again most of the illustrations in text books are of objects which have been carefully selected so that they have been sectioned in the most favourable orientations usually either exactly in transverse section (T.S.) or in longitudinal section (L.S.). In practice such appearances are rare and often all possible orientations are presented in a section, a fact which it is essential to bear in mind when trying to interpret any section. For example Fig. 3.1 shows some of the appearances which might be expected from the sectioning of a sheet of columnar cells in various planes. Similarly, the appearance of a duct lined with cuboidal cells or hollow spherical vesicles will depend upon the plane in which they are cut. Often this extrapolation from the sectioned appearance is very difficult and then graphic reconstruction techniques have to be employed. In this case serial sections are prepared and the outlines of the particular organ as they appear in each section are drawn onto sheets of thin wax or plastic. These outlines may then be cut out and each superimposed on its neighbour, carefully orientated with respect to some reference point, to form a solid model of the object.

An alternative approach, much used in the nineteenth century but unjustly neglected nowadays, is to separate the various parts or cells by means of dissociation techniques. This allows the shapes of individual cells to be appreciated very easily.

Thinking of the three-dimensional spatial structure is very essential, but this forms only a part of the study of a cell or organ. Differences in appearance due to the physiological state of the organism must also be considered as well as the changes due to the natural processes of ageing in the animal. This is particularly true, for example, with respect to gland cells whose appearance will largely depend upon their stage in the secretory cycle. Again, marked changes in appearance may result from differences in the degree of distension of the vessels at the time

of death and upon whether there has been any noticeable collapse after death. The impression which one gains of the spleen for example depends very largely upon this latter fact. Many organs exhibit markedly different histological appearances according to whether they have been taken from a young or an old animal; the most obvious examples are to be found in the female reproductive system. Here the appearance of the ovary and uterus of the old animal is completely different from that of the same organs taken from a young female (compare, for example, Figs. 24.17 and 24.19). Discernible changes do however occur in other organ systems of the body.

All surfaces, whether internal or external, are covered by a layer of epithelial tissue which may arise from any of the three primitive germ layers (ectoderm, mesoderm or endoderm). An epithelium consists of cells of various shapes held together with a minimal amount of intercellular substance, and appears as continuous sheets covering the surface, or lining the internal

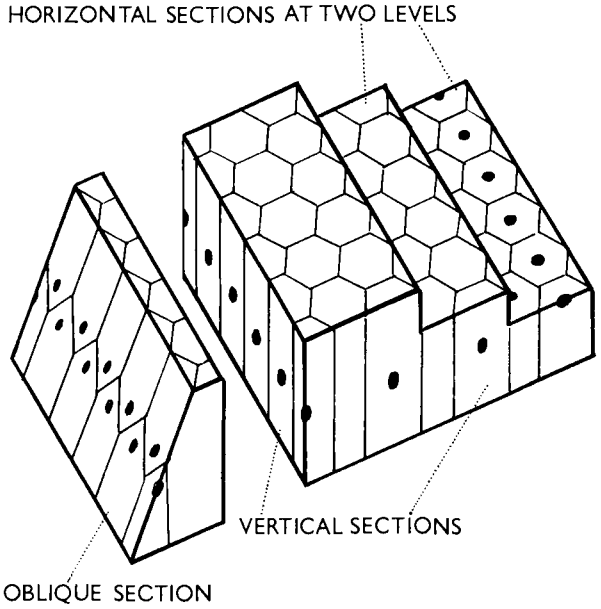


FIG. 3.1. A diagram to show the appearances which may be obtained when a sheet of simple columnar epithelium is sectioned in various planes. Notice how an oblique section may give the false appearance of two layers of cells and how some vertical sections may show some cells to be without nuclei. Such artifacts of sectioning geometry must be allowed for when mentally reconstructing the form of a tissue from microscopic sections.

cavities of the body or invaginated tubes and sacs; occasionally it appears as solid cords which have lost their original relation to a free surface (e.g. interstitial cells of the ovary). The individual cells may vary in shape from flat or squamous to cubical or columnar, and according to their arrangement the epithelium is spoken of as simple (one cell thick) or stratified (several layers thick). The form of the individual cells during life varies greatly in accordance with the changes due to stretch or contraction of the surfaces that they cover, the variations being greatest in the last group.

Epithelia serve many different functions in the body and this is reflected in their morphological specializations. Epithelia line or cover all surfaces of the body and so form the principal barrier between the organism and its environment, hence their primary function is one of protection. This may be mechanical, helping resist trauma, but also protection with respect to moisture loss is very important. Again an epithelium serves to protect the body tissues against possible damage by the hypertonic and acid urine contained in the bladder.

Many epithelia however fulfil other roles as well. Because of their content of nerve endings they serve as general sensory surfaces. The skin, especially, serves for touch and temperature discrimination; often more specific neural elements are found in epithelia to serve as chemoreceptors, the obvious example being the olfactory hair cell in the nasal mucosa. It is obvious also that as every body surface is covered by epithelium they must play an important part in absorptive and secretory processes. In the latter case the whole epithelium may serve as a gland, each cell being secretory (e.g. the gastric surface epithelium) or only isolated cells in the epithelial sheet may function in this way (e.g. mucous secreting goblet cells). In this example the functional specialization has led to a structural specialization and the "goblet" appearance makes this type of cell easily recognizable in a histological preparation (see Fig. 1.6).

Most of the specializations are reflected in changes which are visible with the optical microscope but in some cases changes occur which are only perceptible with the electron microscope. Some of these will be considered in more detail later.

(1) **Simple epithelia**

(*a*) **Squamous ("Pavement") Epithelium.** The cells are thin and plate-like, spread over the underlying connective tissue surface and adhering closely together. In surface they present a

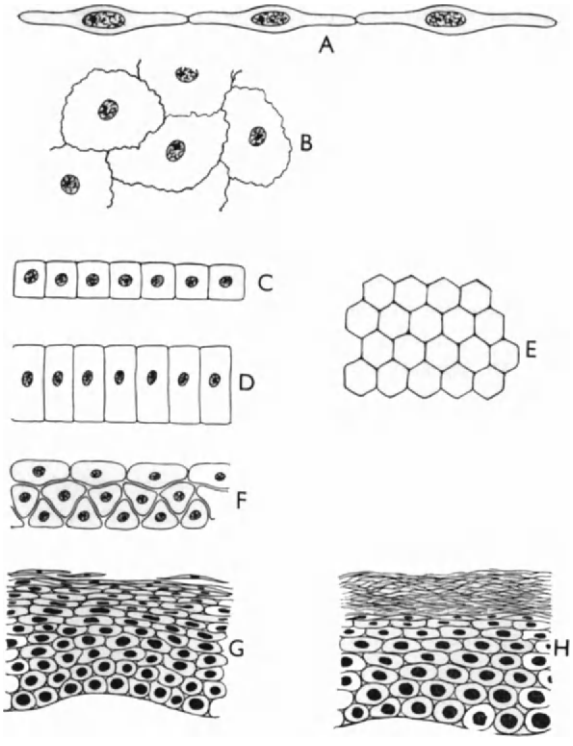


FIG. 3.2. A diagram of the various types of epithelium; (A) squamous, seen in section (B) squamous, seen in face view (C) cuboidal (D) columnar (E) cuboidal and columnar in face view (F) transitional (G) stratified squamous non-keratinized (H) stratified squamous keratinized.

mosaic appearance and their cell boundaries appear to interdigitate in a sinous manner (Figs. 3.2A and B, 3.3A). The cells are often so thin that the central nucleus produces a bulge in the cell. E/M studies have shown the presence of large numbers of vesicles throughout the cytoplasm appearing to originate from the plasma membrane. In endothelial cells of some blood vessels apparent thin places are seen, where the plasma membrane of the two opposite

surfaces of the cell becomes apposed. These are sometimes called "pores" or "fenestrae"; they have a diameter of about 30–50 nm. and are frequent in the endothelium of capillaries in kidney, gut and endocrine glands (Fig. 13.13). The presence of vesicles and "pores" together with experiments using electron-dense markers suggest that this type of epithelium is active in the transport of substances across the cell.

Chief Distribution

The thin limb of Henle's loop in the kidney.
Smallest ducts of some glands.
Inner surface of tympanic membrane and of membranous labyrinth of ear.
The alveolar lining of the lung.
Mesothelium lining serous cavities.
Mesenchymal epithelium lining cavities in connective tissue.
Endothelium lining blood and lymph vessels.

(The last three groups consist of simple squamous cells but they are sometimes regarded as different from the simple pavement epithelium because of their functional reactions.)

(*b*) **Columnar and Cubical Epithelium** (Figs. 3.2, 3.3). These two categories are often regarded as separate types, but in fact any difference is more of degree than of kind, as the appearance of the cells may vary so much with the physiological state and with the state of distension of the organ. The cells are usually tall and prismatic and are arranged in a single layer on what was formerly called the "basement membrane". The E/M has shown that this actually consists of a complex of a thin layer of mucoprotein immediately underneath the cells (this is termed the *basal lamina*) together with an outer layer of collagen or reticular fibres embedded in an amorphous matrix. When seen from the surface the epithelial sheet presents a mosaic of hexagonal areas. The cells vary in height and often contain secretory granules. Sometimes the cell is completely transformed by the accumulation of large amounts of secretion as in goblet cells and often the free surface bears cilia or microvilli. The nucleus tends to be located towards the base of columnar cells and centrally in the more cubical cells.

If the cells are very closely packed and very tall, then only the tallest may actually reach the lumen, giving rise to what is termed a *pseudostratified* epithelium. All the cells in such an epithelium, however, retain an attachment to the basal lamina. Those cells which are basal in situation may be regarded as reserve cells; some of these eventually grow and reach the lumen whilst others undergo mitosis to provide a continuous succession of new basal cells. The pseudostratified epithelium may easily be recognized in sections as the nuclei appear to be on several levels (Fig. 3.4) whilst in a simple columnar epithelium all the nuclei tend to be situated at one level.

Often a columnar epithelium is further subclassified according to the nature of the luminal surface, e.g. the presence of cilia.

Chief Distribution

(i) *Typically cubical*

Germinal epithelium of ovary.
Lining of vesicles in the thyroid gland.
Choroid plexus.
Excretory ducts of many glands and secretory alveoli of many glands (in this case the cells often are compressed and assume a pyramidal shape).
Inner surface of lens capsule.
Pigment layer of retina.

(ii) *Typically columnar*

Lining of alimentary canal from stomach to rectum.
Gall bladder.

(iii) *Ciliated columnar*

Ependyma of spinal cord and the ventricles of the brain.
Endometrium of the uterus (in part).
Uterine tubes (in part).
Small bronchi and bronchioles.

(iv) *Pseudostratified*

Trachea and large bronchi.
Pharyngo-tympanic (Eustachian) tube.
Vas deferens.
Male urethra (membranous and penile portions).

(2) Stratified Epithelium

The cells of stratified epithelium are arranged in many layers, of which the deepest rests on the basal lamina (Fig. 3.2). In sections cut at right angles to the surface the shape of the cells is characteristic: the deepest layer consists of columnar cells frequently showing mitosis: nearer the surface the cells are more polyhedral or cubical, gradually becoming flatter until the squamous surface cells are reached. The cells of the intermediate layers are separated by fine channels containing inter-cellular matrix and tissue fluid derived from the blood: these spaces are bridged across by fine protoplasmic processes which come into apposition with the processes of neighbouring cells. Well developed desmosomes are often present between these cell contacts, and it has been suggested that these represent a functional adaptation for the maintenance of a high degree of strength and structural integrity in the stratified epithelium. Such an epithelium is found wherever the part is subject to friction; as the outer cells are rubbed off they are replaced from below. In practice it is usual to distinguish two subdivisions of stratified epithelia; one—non-keratinized—is found covering surfaces which are not subject to dessication whilst the keratinized variety is present on exposed skin surfaces.

Non-keratinized stratified epithelium (Fig. 3.5A) is characterized by the fact that all its cells are living and hence in sections the nuclei and outlines of the cells can easily be seen. The typical developmental sequence of changes described above is clearly visible, the cells passing from a rounded form to a flattened squame as they become more superficial. Keratinized epithelium (Fig. 3.5B), on the other hand, is typified by the fact that the cells die off as they become keratinized. It is this protein which confers its characteristic toughness and water resistance on this type of epithelium; as the keratin develops a definite division of the epithelium into layers may be seen. These are most clearly developed in some regions of the skin (see Chapter 17). As the deeper cells divide, the resulting cells are pushed upwards and become flattened. As they become further and further removed from the basal layer granules of keratohyalin accumulate within their cell bodies and these granules eventually become compacted into the clear homogeneous mass of eleidin which in turn becomes transformed into the true keratin.

Keratinized epithelium may easily be recognized in histological sections by the presence of the superficial detaching flakes of keratinized squames, by the acidophilic staining reaction of the keratin itself and by the lack of discernible nuclei and cell boundaries in the superficial layers.

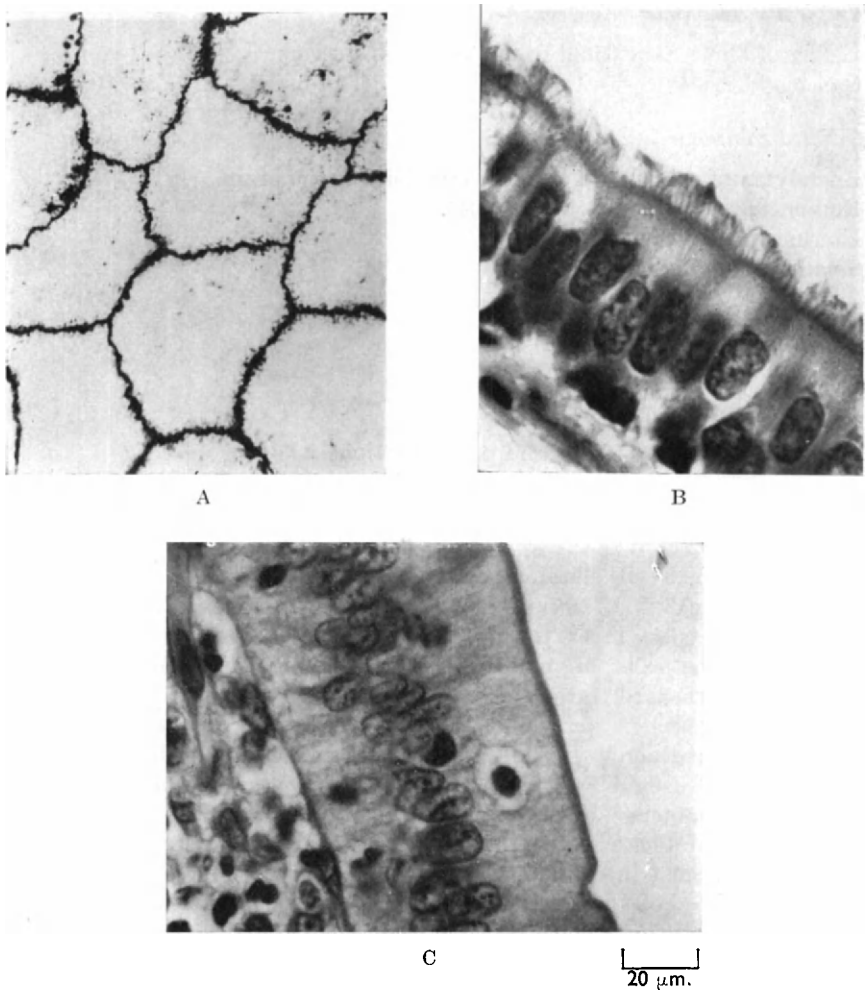


FIG. 3.3. Photographs of various types of epithelium; (A) squamous in face view. The matrix between the cells is heavily stained but the nuclei of the cells are not visible. (B) pseudo-stratified ciliated (trachea) (C) columnar (gut lining) (D) cuboidal (kidney collecting duct) (E) transitional (bladder).

Chief Distribution

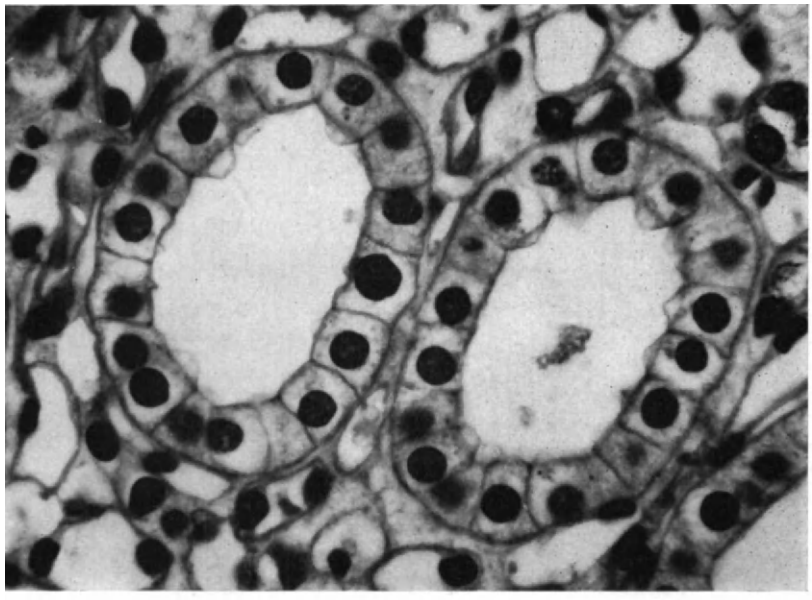
(i) *Non-keratinized*

Lining of mouth, pharynx and oesophagus.
Vagina.
Cornea.

(ii) *Keratinized*

Epidermis covering the whole surface of the body.

Transitional epithelium (Fig. 3.3) is a variety of stratified epithelium found lining the urinary tract. All the cells are living, and the surface layer is not squamous: in this respect it differs from stratified epithelium. It allows of great alterations in extent and thickness. The deepest cells are usually columnar or cubical, the more superficial cells are irregularly poly-



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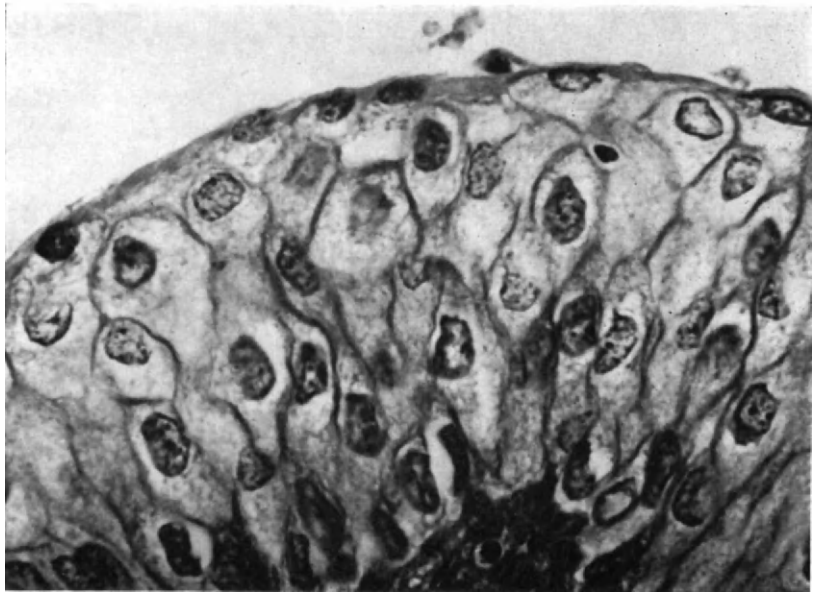


E

20 μm.

FIG. 3.3—*continued.*

hedral or "pear-shaped", and the surface layer consists of large cells, often binucleate, of "umbrella" shape, the concavity fitting over the cells in the next deeper layer.

Extension of the epithelial area, due to stretching, is probably largely mediated by changes in the shape of individual cells which become long and flattened. The E/M has shown that this type of cell is characterized by the presence of many fine filaments in the cytoplasm, particularly near the luminal surfaces of the most superficial cells. The work of R. M. Hicks has

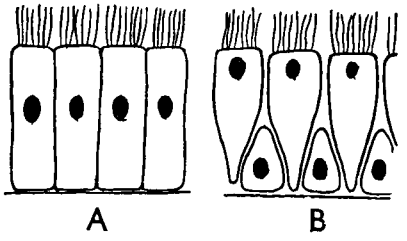
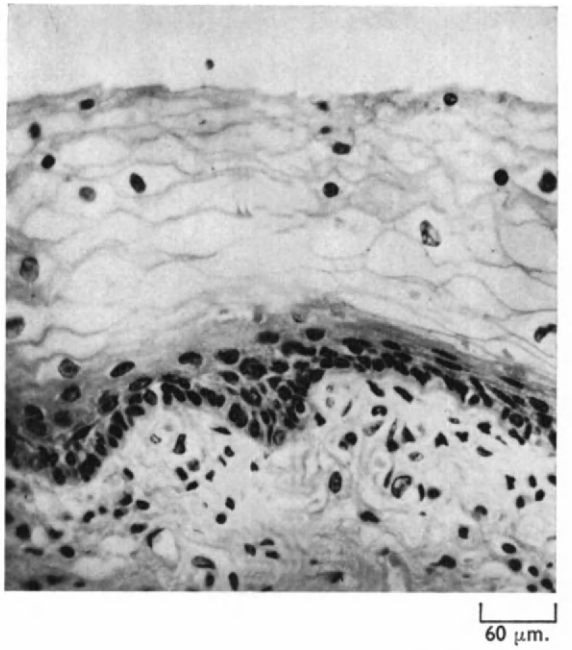


FIG. 3.4. A diagram of columnar epithelium (A) and pseudostratified epithelium (B); notice the small basal cells in (B) do not reach the lumen, and that the appearance of the nuclei would be that of two separate rows.



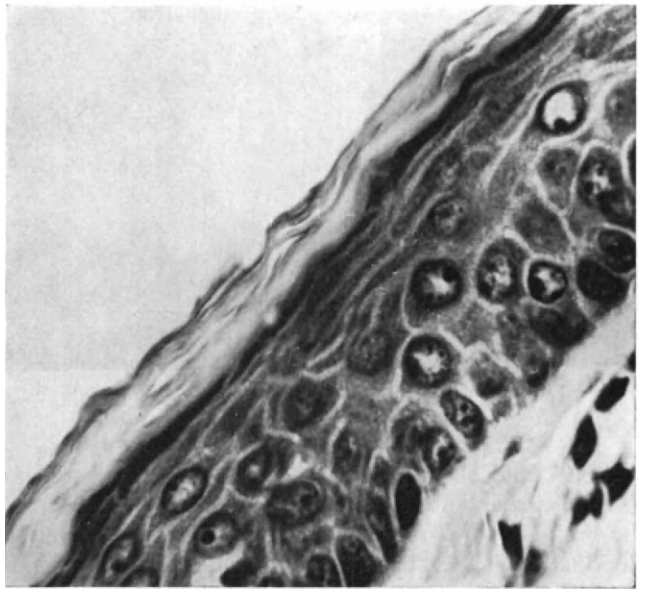


FIG. 3.5. (Above) stratified non-keratinized epithelium from the vagina. (Below) skin, a typical keratinized epithelium. Cell nuclei and outlines cannot be recognized in the keratinized layers.

shown a peculiar thickening of the outer lamina of the unit membrane of the luminal surface of these same cells. This is interpreted as representing an incipient keratinization in order to provide resistance to the penetration of urinary constituents. The superficial cells are also closely linked to each other by well developed junctional complexes (see p. 40).

Chief Distribution

Pelvis of the kidney.
Ureters.
Bladder.
First part of the urethra.

The Functional Surfaces of Epithelial Cells

The epithelial cell presents three surfaces with different functions.

(*a*) A basal surface, usually resting on a basal lamina and attached to the underlying connective tissue.

(*b*) Lateral surfaces, adjacent to surrounding cells of the epithelium and separated from them by intercellular substance.

(*c*) A free or luminal surface, which may undergo very varied modifications.

(i) *Basal Surface and Basal Lamina.* The basal lamina is a thin layer between the epithelium and the underlying connective tissue; it is usually a product of the connective tissue formed

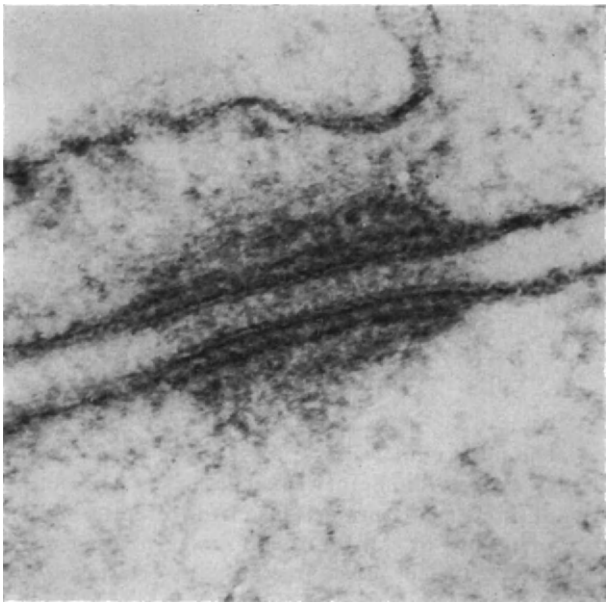


FIG. 3.6. An electron micrograph of a desmosome. The typical thickening associated with the plasma membrane is apparent, as well as the plaque of electron-dense material in the cytoplasm and in the interstitial matrix. 0·1 μm.

as a condensation of its intercellular substance at the epithelial surface of contact. Generally associated with the basal lamina is a mesh of very delicate fibres known as reticular fibres (see Chapter 6). Treatment of tissue sections with periodic acid followed by Schiff's reagent (the PAS technique) results in a reddish purple colouring of basal laminae indicative of the

presence of polysaccharide material, probably associated with the amorphous condensed intercellular substance. The basal lamina and the associated amorphous substance and the fibres form the "basement membrane" as described by optical microscopy. In the thyroid gland and urinary passages no basement membrane appears visible with the optical microscope but the E/M has shown that there is a thin basal lamina present. The most common specialization noted in the basal surface of epithelial cells is the presence of a series of infoldings of the cell plasma membrane. Such infoldings usually have associated mitochondria in the cytoplasm and are thought to be concerned with rapid transport of ions. Examples are found in the cells of the distal convoluted tubule of the kidney and in the so-called "striated" ducts of some salivary glands.

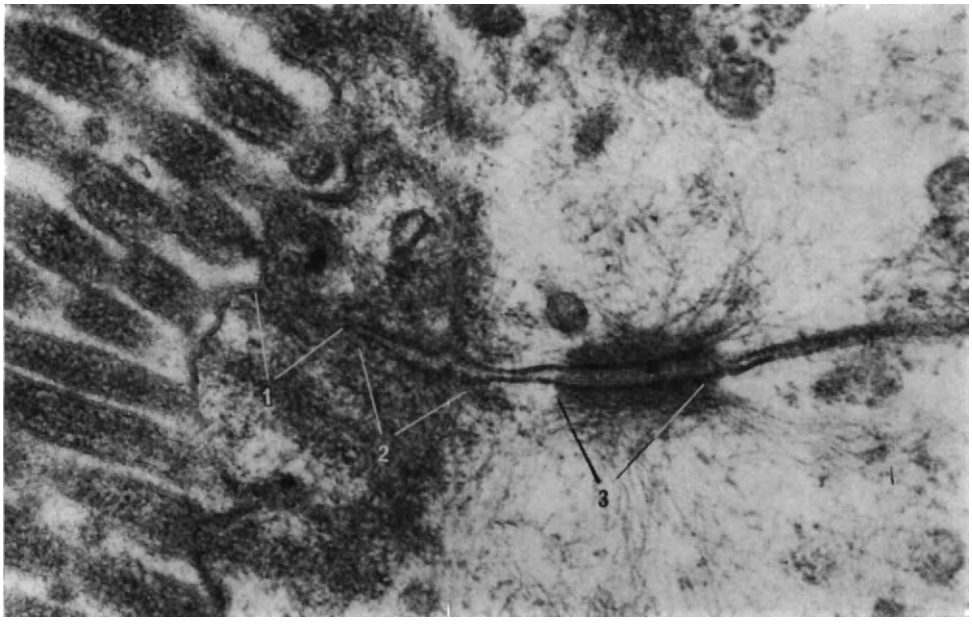


FIG. 3.7. A junctional complex between two proximal convoluted tubule cells of the kidney (1) the zonula occludens (2) the zonula adhaerens (3) the macula adhaerens. 0·1 μm. The trilaminar appearance of the unit membrane may be seen in the microvilli at the left of the micrograph.

(ii) *Lateral surfaces.* The intercellular substance is scanty and cohesion of the cells, which is very marked in such places as the alimentary canal and kidney collecting tubules, is probably assisted by the presence of interlocking projections of the lateral cell membranes (Fig. 20.19). In other situations (e.g. epidermis of skin), in regions where adjacent cells are in very close contact, a localized thickening of the inner lamina of the cell membranes occurs, together with a condensation of cytoplasmic matrix and a convergence of cytoplasmic microtubules on the region. At these points, the *desmosomes* or *maculae adhaerens* (Fig. 3.6.), there is firm cohesion between the cells and possibly enhancement of intracellular transport mechanisms. Studies with the optical microscope showed a darkly staining structure in between cells of columnar epithelia towards the lumen. This was called the *terminal bar*. The E/M has shown that this region in reality contains three separate specializations termed by Farquhar and Palade the *junctional complex* (Fig. 3.7); the three regions are:

(i) the zonula occludens—a region of fusion between the plasma membranes of adjacent cells;

(ii) the zonula adhaerens—a region of close apposition of the plasma membranes;
(iii) the macula adhaerens or desmosome.

Both the zonula occludens and the zonula adhaerens are thought to extend as a continuous band round the periphery of the cell, whilst the desmosomes are distributed in a discontinuous array beneath these. The junctional complex is regarded as important in maintaining the integrity of the surface layer of epithelium and in preventing any substances passing from the lumen into the tissues via the intercellular space.

Occasionally no limits can be seen between the cells, and the epithelial sheet then becomes a *syncitium*: such a condition is found in the covering layer (the syncitiotrophoblast) of the trophoblastic villi of the developing embryo.

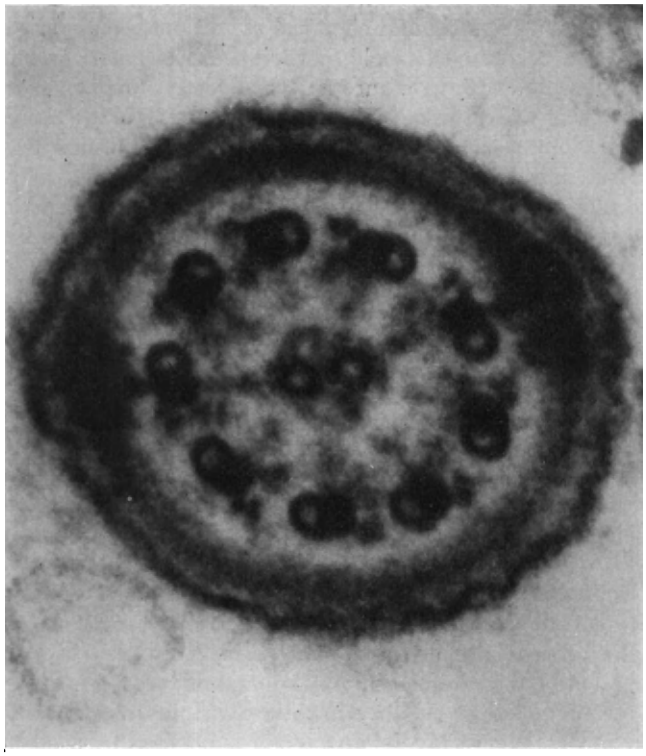


FIG. 3.8. An electron micrograph of a transverse section through the principal piece of a mammalian spermatozoon. This shows the central doublet and the nine double peripheral fibres of cilia. An extra outer sheath is present in spermatozoa which is not present in other cilia. 0·10 μm.

(iii) *The Free or Luminal Surface.* The cells lining the small intestine are provided with a border that appears under the optical microscope as fine striations at right angles to the surface (p. 306). The electron microscope shows that this "striated or 'brush' border" consists of large numbers of fine projections of the cytoplasm, in parallel array and all of the same length. The projections, which are covered with the cell membrane and with the glycocalyx, are known as *microvilli* and their frequent association with cells having an absorptive function suggests that they are a device for increasing the surface area of the cell (Fig. 2.1) as an adaptation for the better absorption of digested food materials.

The free surface of the cells of the proximal convoluted tubules of the kidney (p. 339) also possess microvilli, and both here and in the small intestine the "brush border" contains the

enzyme alkaline phosphatase. This may be concerned in the mechanism whereby sugars such as glucose are transferred across the cell membrane.

Many epithelial cells develop *cilia*, thin hair-like processes of varying length. Recent investigations show that cilia possess a rather characteristic ultrastructure, which they share with flagella and the tails of spermatozoa. They contain nine pairs of longitudinal strands arranged in a ring with a single pair of apparently similar structures placed at the middle of the ring (Fig. 3.8). That these nine paired filaments are concerned with the motile mechanism seems almost certain, but the nature of the part they play is still a matter for speculation. The cilia continue into the substance of the cell, the roots springing from the region at the side of the nucleus, usually in association with the basal body, a structure homologous with the centriole. Cilia are usually motile, always bending stiffly in one direction with a quick beat and recovering limply and more slowly. The movement travels in waves along the surface. Ciliary activity is affected by physical and chemical conditions, being accelerated by warmth and by calcium salts, and decreased by cold, carbon dioxide, ether vapour and chloroform: this latter factor may become of importance in the defective expulsion of mucus from the respiratory passages during anaesthesia. Ciliary movement is always associated with movement in a fluid: in the oviduct the ovum is propelled towards the uterus, in the respiratory passages mucus is driven spirally upwards towards the mouth carrying trapped dust particles with it: in the vasa efferentia the sperms are assisted towards the epididymis. Some cells carry non-motile "stereocilia": the E/M has shown these to resemble microvilli rather than cilia. In the epididymis these help in eliminating the secretion from the cells, in the maculae and cristae of the ear they are concerned in receiving vibratory stimuli.

True *cuticles* covering the surface of an epithelium like a sheet are rare in the human; the enamel of the teeth and the tectorial membrane of the cochlea are the most obvious examples.

Blood Supply. An epithelium usually has no direct blood supply, but obtains its nourishment from the fluid in the intercellular spaces which has originated from the blood vessels of the underlying tissue. An exception is the stria vascularis of the cochlea (p. 424).

Nerve Supply. Free nerve fibres are often found penetrating between the deeper parts of the cells of a simple epithelium. A stratified epithelium is usually supplied with a sub-epithelial nerve plexus from which free fibres arise to penetrate the epithelium and ramify among the deeper layers of cells (Fig 11.20).

Regeneration of Epithelia. All epithelia show marked regenerative power. In the wear and tear of everyday life certain epithelia, such as those of the epidermis, may frequently be damaged and shed: this process is well marked in the skin as the outer layers undergo cornification and the resulting dead flakes are continually rubbed off. Any physiological epithelial loss is made good by a proliferation of cells by mitotic division. In a stratified epithelium the mitoses occur chiefly in the deepest layers, and repair is effected by a pushing up of the cells from below. In the alimentary tract the new cells are provided by division of undifferentiated epithelial cells at the base of the gastric or intestinal glands: these cells become gradually moved up as those nearer the surface are shed.

After local injury all types of epithelial cells can respond by mitotic division, the resulting cells maintaining the specific character of the original epithelium. In an effort to cover rapidly an area denuded of its epithelium, the remaining cells may flatten and show a gliding movement towards the damaged area even before regenerative division begins.

Endothelium and Mesothelium

Endothelium and mesothelium are the names given to membranes similar to other epithelial membranes which arise entirely from the mesoderm.

(1) **Endothelium.** This includes only the simple squamous epithelium lining the heart, the blood vessels (see Ch. 13) and the lymph vessels. Endothelium arises through flattening of the mesenchyme cells.

(2) **Mesothelium.** This includes the layer of squamous cells lining the coelom and covering the surface of all *serous membranes* (pleura, peritoneum, pericardium, and the serous coat of the scrotum). Under certain conditions, such as inflammation, these cells may give rise to typical fibroblasts. Regeneration of damaged mesothelium is by mitosis of its cells. E/M studies have shown the presence of numerous short microvilli on mesothelial cells.

Glands

One general function of epithelial tissue is that of secretion, in other words, the absorption of materials from the medium in contact with the cell and the elimination of other materials into the same or some other medium. For this function specialization of the epithelium into glands occurs: of these glands some, the *exocrine* ones, eliminate their secretion on to a surface either directly or by means of a duct, while others, the internally secreting or *endocrine*

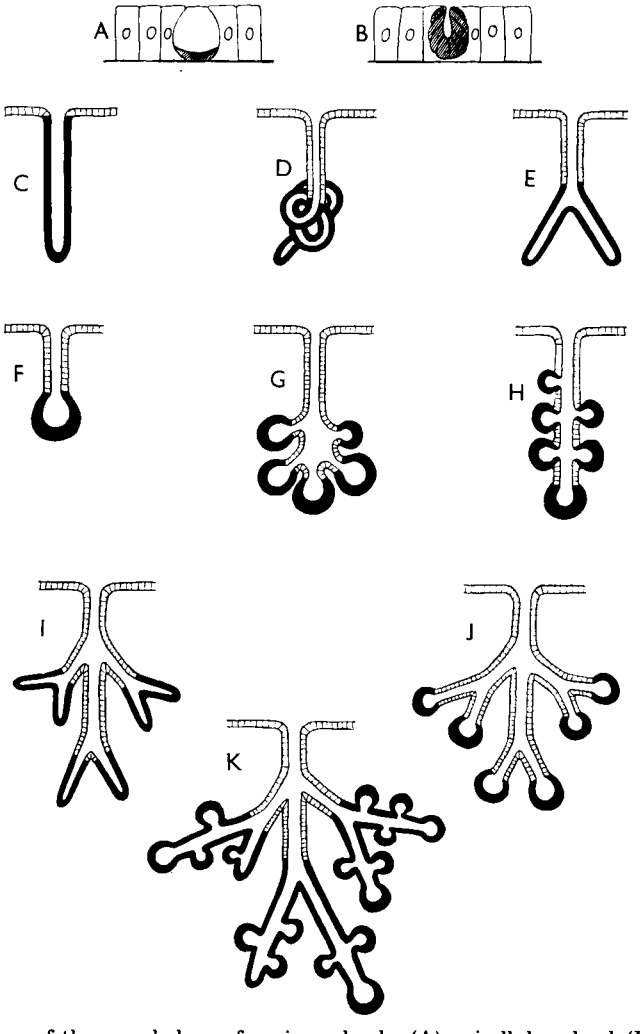


FIG. 3.9. A diagram of the morphology of various glands; (A) unicellular gland (B) simple multicellular gland within the thickness of the epithelium (C) simple tubular gland (D) simple coiled tubular gland (E) simple branched tubular gland (F) simple alveolar gland (G) and (H) two types of simple branched alveolar gland (I) compound tubular gland (J) compound alveolar gland (K) compound tubulo-alveolar gland. The secretory tissues are in black.

glands, pass their secretion directly into the blood or lymph. The method of release of the secretory product from the gland cell is sometimes used to classify glands. In the *merocrine* type of secretion, the product is released by a process of exocytosis without the loss of any glandular cytoplasm. This secretory type is found in the majority of glands; for examples the pancreas and the salivary glands may be cited. If, however, the secretion accumulates in the apical part of the cell which is later pinched off to release the product then the mode of secretion is called *apocrine*. A good example is the lactating mammary gland. Occasionally the whole of the cell, together with its accumulated secretion, is shed and then the gland is said to be of the *holocrine* type. The sebaceous glands are good examples of holocrine secretion and it is obvious that this type of gland must have a high mitotic rate amongst the secretory cells in order to replace those lost during the process of secretion.

In histology a further classification of glandular tissues which is often of use is according to the nature of their secretion, i.e. whether it is mucous, or serous or a mixture of both. Mucous gland cells can be recognized by their acidophilia, their "foamy" or empty cytoplasmic appearance and by the flattening of the nuclei towards the base of the cell. Serous cells usually have a basiphilic cytoplasm, regular nuclei in the basal portion of the cell and show the presence of the secretory granules in the apical part of the cytoplasm. The E/M shows serous cells to have a large quantity of regularly-arranged rough surfaced endoplasmic reticulum in the basal region of the cell.

Exocrine glands may be unicellular, like the mucin-secreting goblet cells, or they may be multicellular; these latter vary from the simple invaginated tube such as the intestinal glands, to the highly complex gland consisting of a terminal glandular portion of secreting alveoli (or acini) and a proximal excretory duct, as in the salivary glands (see diagram Fig. 3.9).

The various glands are described under the organs to which they belong.

All glands contain some loose connective tissue, and compound glands are surrounded by connective tissue that forms a *capsule*: from the capsule connective tissue *trabeculae* extend into the gland, dividing it into lobules. The glandular tissue of a gland is called the *parenchyma*, and the connective tissue is the *interstitial tissue* or *stroma*. (The term *stroma* is used to denote the connective tissue groundwork of any organ or complex tissue.) The blood vessels usually form dense networks immediately below the basal lamina on which the epithelial cells rest, while the lymph capillaries are found in the interstitial connective tissue. The blood vessels generally possess vasomotor nerves and, in addition, there is a plexus of secretory nerve fibres below the basal lamina: this plexus often sends branches to penetrate to the bases of the gland cells where a second plexus is formed; nerve fibres derived from this latter plexus end freely between the cells.

Mucous Membranes

A mucous membrane lines every cavity or canal that opens to the exterior, i.e. the alimentary tract, the respiratory tract, and the urino-genital tract. It consists of a surface of epithelium resting on a basal lamina and an underlying connective tissue, or *corium* (*lamina propria*). The surface may be increased by simple glands: frequently many mucous glands are present, but sometimes (e.g. bladder) there are none. Sometimes the mucosa is separated from the underlying submucosa by a layer of smooth muscle, the *muscularis mucosae*: this occurs in the alimentary tract.

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CHAPTER 4

BLOOD AND LYMPH

BLOOD

Blood is a unique tissue which consists of a fluid plasma (the intercellular or interstitial substance) in which float the blood corpuscles. The total volume of blood in the adult is about 5 to 6 litres and represents approximately 7 per cent of the body weight. The blood is the chief transport system of the body; it is essential for the movement of metabolites, oxygen and carbon dioxide, hormones, and antitoxins and antibodies, to name but a few. Both the cells and the plasma play an important role in these transportation functions. Oxygen for example

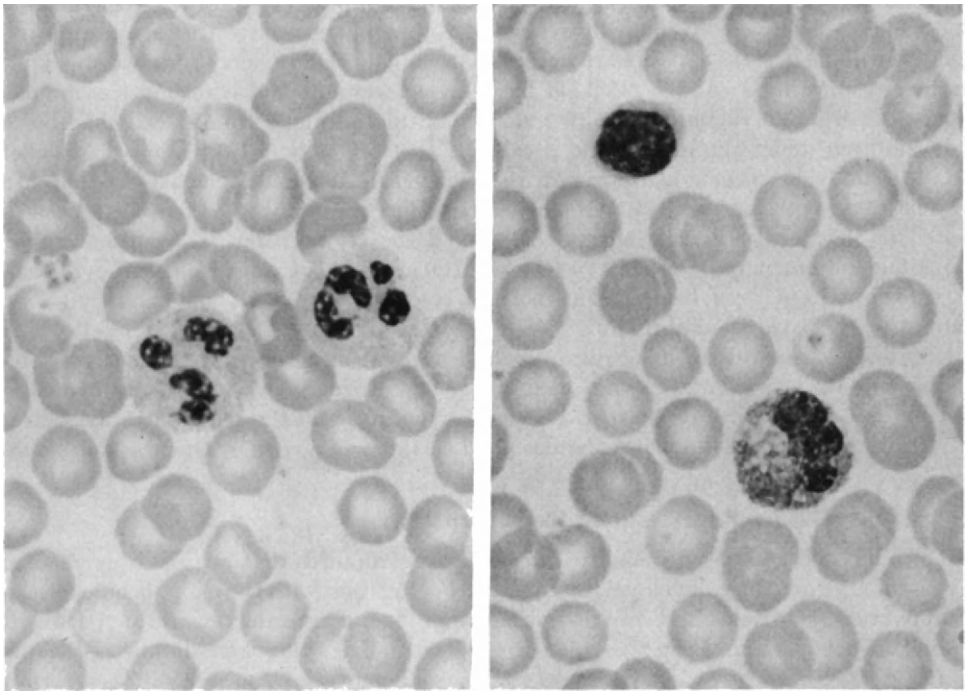


FIG. 4.1. The appearance of a normal stained blood film. In addition to the erythrocytes may be seen (from left to right) a clump of platelets, two polymorph neutrophils, a single small lymphocyte and an eosinophil.

is circulated inside the red cells in loose combination with their contained haemoglobin whilst some of the carbon dioxide and cell excretory products and the hormones circulate in solution in the plasma. Because the plasma is almost entirely water, it plays a vital part in the thermoregulatory processes of the body. Blood is a mobile tissue in that it may be rapidly transferred from one part of the body to another and its pattern of distribution may be rapidly changed by alterations in the state of constriction of the distributing vessels; this means that heat may be transferred from its site of production deep in the body to the surface where it may

easily be dissipated if the need arises and vice versa. The high specific heat and high thermal conductivity of the water which largely composes the plasma are very significant in this respect.

The presence of active cells circulating in suspension in the plasma of the blood is important in the defence mechanisms of the body. Invading micro organisms may be phagocytosed by the neutrophils whilst the lymphocytes are of vital significance in the processes of antibody transport and the production of cellular immunity.

The formed elements normally present in the plasma consist of:

(1) Erythrocytes, or red corpuscles, whose pigment (haemoglobin) gives the blood its red colour.
(2) Leucocytes, or white blood cells.
(3) Platelets.

The relative proportions of the volume of corpuscles and plasma may be determined by the use of the *haematocrit*. If blood to which an anticoagulant has been added is allowed to sediment in a special narrow tube, three layers will form. The lowest layer, dark red in colour, contains the erythrocytes and occupies about 45 per cent of the volume. Above this is a thin grey or cream-coloured layer (known as the "buffy coat") which represents the 1 per cent of the total volume which is occupied by the leucocytes and the platelets. Above this again appears the clear yellowish plasma. It is possible to determine the relative numbers of the various kinds of corpuscles by diluting a sample of blood by a known amount and counting the number of each type of corpuscle visible in a known volume of diluted blood; the special chamber used for this counting under the microscope is called a *haemocytometer*. The haemoglobin content of the blood may be measured colorimetrically using an instrument called a *haemoglobinometer*. The ratio between the haemoglobin content and the number of red corpuscles (both expressed as percentages of the normal) is termed the *colour index*. The normal value for haemoglobin content may be taken as 14·8 g per 100 ml., and the normal erythrocyte count as 5,500,000 per cu. mm. (see below). The normal colour index is 1, but values of 0·9 and 1·1 cannot be regarded as abnormal.

The cytological details of leucocytes are best studied in a thin dried smear preparation stained by a neutral stain known as a Romanowsky-type stain. Such stains consist of a complex formed from the ionic form of an acid dye (usually eosin$^-$) and the ionic form of a basic dye (usually azure$^+$). The best known examples are Leishman's, Wright's, Giemsa and MacNeals stains.

Erythrocytes (Red Blood Corpuscles) (Figs. 4.1, 4.3)

The red blood corpuscles are highly differentiated structures that can easily be recognized in any tissue: during their development their nuclei, together with the endoplasmic reticulum, Golgi apparatus, centrioles and mitochondria are all lost. Erythrocytes are bi-concave discs with thickened edges, appearing circular when seen in face view and dumb-bell shaped in section (Fig. 4.3). They have a tendency to stick together by their concave surfaces, forming a rouleau, like a pile of coins (Fig. 4.4): this may occur in the circulating blood stream in places where the current is not very rapid. A phenomenon known as "sludging", where the erythrocytes form irregular clumps in the lumen of the vessel, is well known in some diseases. A single erythrocyte is pale yellow, but when the corpuscles are seen in a mass the haemoglobin in the corpuscles gives the characteristic red colour of blood. The haemoglobin within each erythrocyte also accounts for the strong acidophilic staining reaction of erythrocytes in blood films and in histological sections. The average number of red corpuscles is 5,500,000 per c. mm. of blood, but the number varies with different physiological and pathological conditions. For example, continued living at high altitudes causes increase in the numbers of circulating erythrocytes of up to 30 per cent. Women usually have a slightly lower total red cell count (4,800,000 per c. mm.) than men, and their haemoglobin percentage is on the average slightly

lower also. The erythrocytes are soft-bodied and very flexible and elastic, readily distorted mechanically but recovering shape immediately: this is easily seen under the microscope by watching blood flowing through a capillary bed (such as in the web of the frog's foot) where the erythrocytes can pass only in single file.

The size of the corpuscles is very constant, the normal range of mean diameter in the dry film being 6·7 μm. to 7·5 μm. (normal mean, 7·2 μm.): in the living state the diameter is about 7·5 μm. to 8 μm.: there is a diurnal variation, the mean diameter in the morning being 0·5 μm. less than the evening diameter. A cell of such "normal" size is known as a *normocyte*: any cell of greater diameter is spoken of as a *macrocyte*, and one of smaller size as a *microcyte*. If there is much variation in cell size *anisocytosis* is said to exist.

Although the erythrocyte lacks all the typical cell organelles, it is not biochemically inert; considerable energy is needed to provide for the maintenance of osmotic pressure differences across the unit membrane which bounds the erythrocyte and to maintain the haemoglobin in

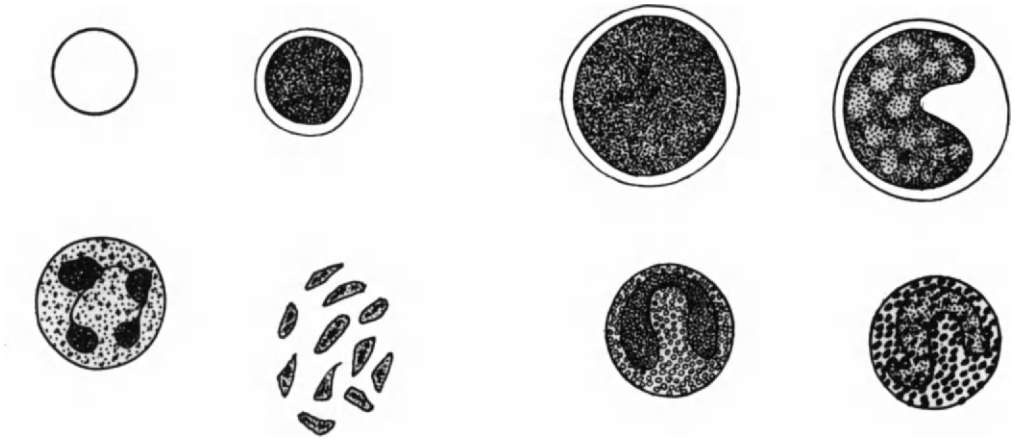


FIG. 4.2. The formed elements in the blood. These are all drawn to the same scale and are (top row, from left to right) erythrocyte, small lymphocyte, large lymphocyte, monocyte (bottom row, from left to right) polymorphonuclear neutrophil, blood platelets, eosinophil and basiphil.

the reduced state (so preventing its oxidation to the inactive methaemoglobin). This energy is derived from the anaerobic glycolysis of glucose to lactic acid and hence all the relevant enzyme systems for this process are present in the erythrocyte.

Functions of Erythrocytes

The red corpuscles contain no nuclei and are consequently short-lived; from isotope studies their duration of life has been estimated as being about 120 days. They have no inherent power of movement and are entirely dependent on the fluid plasma for transport; they are normally never found outside the blood stream. The number in actual circulation varies with the requirements of the body: large numbers can be stored temporarily in the spleen and sent out again when necessary. New corpuscles are constantly being added from the bone marrow to replace the worn-out ones that are being continually removed. It has been estimated that about 25×10^{10} erythrocytes are lost every day, but it is very rare to see an erythrocyte inside a macrophage. The most probable answer to this apparent paradox is that the erythrocytes become fragile and break up whilst they are in the circulation. The resulting small fragments (known as *haemoconia*) are then removed from the plasma by cells of the reticulo-endothelial system (see p. 78), especially those in the spleen and bone marrow. In this way the iron in the haemoglobin can be reused by the body for the synthesis of new haemoglobin.

Their chief function is the carriage of oxygen; this depends on the haemoglobin they contain. If there is some deficiency of haemoglobin or a reduction in the number of erythrocytes below normal a condition termed *anaemia* results.

The other functions of the red blood corpuscles include helping in the regulation of the pH of the blood, and carrying of the blood group antigens. For further detail, reference should be made to a text-book of physiology.

Effect of External Factors on Erythrocyte Shape

The erythrocytes respond rapidly to alterations in the external medium: this would be expected from a consideration of their functions. They are bounded by a very thin, semi-permeable, elastic membrane that contains lipids and allows of the passage of water and of some water-soluble substances while preventing the passage of others. The normal shape of the corpuscles is seen in an isotonic solution, i.e. one in osmotic equilibrium with the corpuscles,

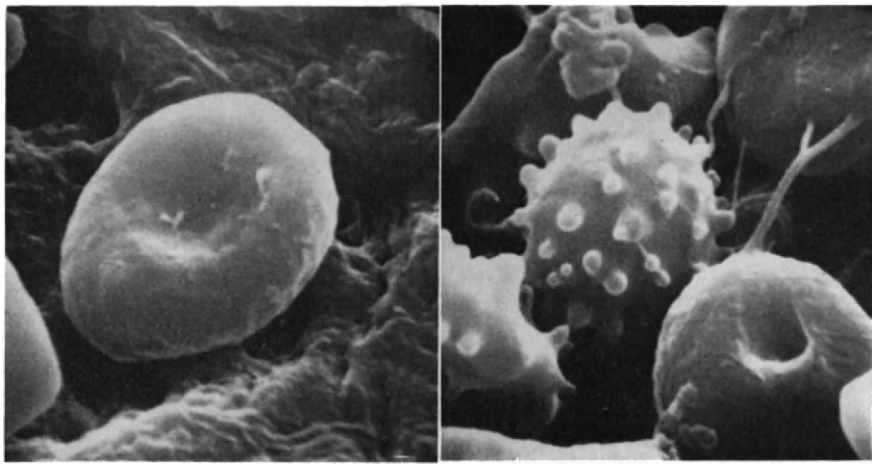


FIG. 4.3. Two scanning electron micrographs of erythrocytes. That on the left is of normal shape and the biconcave disc appearance is well seen; the blood corpuscle in the centre of the right-hand micrograph has become crenated due to loss of water.

such as a 0·9 per cent NaCl solution. In distilled water or hypotonic solutions (e.g. 0·3 per cent NaCl) the corpuscles become spherical owing to the passage of water into them: they lose their colour as the haemoglobin dissolves out into the surrounding fluid: this is called *haemolysis* (or "laking"). If the surrounding medium is hypertonic (e.g. 2 per cent NaCl) the corpuscles become irreparably shrunken and crenated due to the passage of water out of them (Fig. 4.3). It is of the utmost importance that this should be borne in mind when carrying out intravenous injections.

Reticulocytes. When erythrocytes are treated with supravital stains such as cresyl blue a certain small number of the corpuscles are found to possess a very finely granular reticulum, such cells being called *reticulocytes*. In a smear stained with an eosin and methylene blue mixture the ordinary erythrocyte is electively stained with eosin, while the reticulocyte shows a diffuse bluish purple staining, or *polychromasia*. In the adult the reticulocytes represent less than 1 per cent of the total number of erythrocytes: they are more numerous in the blood of the foetus and of infants, and increase in the peripheral blood after haemorrhage: the precursors of the erythrocytes in the bone marrow (see p. 59) show the same characteristic. For these reasons reticulation is regarded as a sign of immaturity; the appearance is probably due

to the deposition of stain on elements of the endoplasmic reticulum which have not yet disappeared from the cytoplasm. The granular reticulum is rich in RNA, which is why the cells are preferentially stained by basic dyes such as methylene blue and thus show polychromasia.

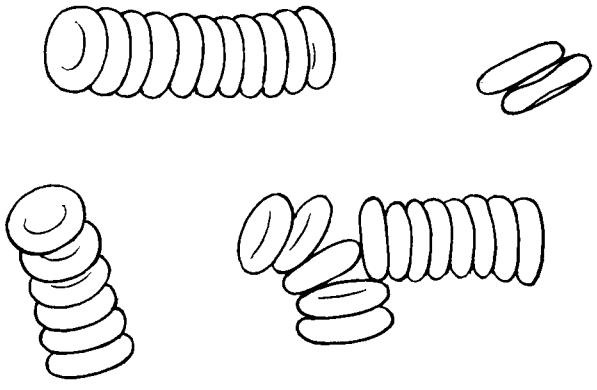


FIG. 4.4. A diagram to show the "rouleaux" formation in erythrocytes.

Leucocytes (White Blood Corpuscles) (Figs. 4.1, 4.2)

The white blood corpuscle contains a nucleus and is therefore a true cell. There is a centriole near the nucleus, but mitosis does not occur in the adult state: mitochondria are present and Golgi material, as in all true cells. Leucocytes are of various kinds and of different sizes. Most of them are phagocytic and have the power of amoeboid movement, and although the blood plasma provides them with a convenient and rapid form of transport, their chief functional activities are shown after they have migrated out of the blood vessels into the surrounding connective tissues.

Their number varies between 4,000 and 10,000 per c. mm. of blood, but the number in circulation shows great variation during the day and in different parts of the body: the number varies from hour to hour and the changes are rapid; consequently white blood cell counts must be regarded as having relative values only. In early life the cells are usually more numerous.

Classification and Morphology of Leucocytes

Leucocytes are divided into two main groups:

(1) Non-granular leucocytes (lymphocytes, monocytes).
(2) Granular leucocytes (polymorphonuclear leucocytes or granulocytes), which contain specific granules in their cytoplasm.

(1) Non-granular Leucocytes

(*a*) **Lymphocytes** (20–25 per cent of the total number) Figs. 4.1, 4.2. These are spherical cells averaging from 6–8 μm. in diameter, although some (the so-called "large" lymphocytes) may be 12–15 μm. in diameter. A complete gradation of sizes exists between these two extremes. The nucleus is spherical but often indented on one side although this may not always be visible in stained films. A single prominent nucleolus can be seen in the living cell. The nucleus is found to occupy the bulk of the cell volume and with Romanowsky staining it colours strongly; the surrounding layer of cytoplasm is coloured a clear blue and some cells exhibit a few azurophil granules in the cytoplasm.

The electron microscope has shown that lymphocytes typically possess few mitochondria, smooth walled vesicles of the endoplasmic reticulum, and few free ribosomes. There is a typical

Golgi apparatus and centrioles are present. Autoradiographic studies indicate that the lymphocytes in the circulation have at least two sources of origin—the lymphoid tissue and the thymus respectively. It seems likely that these two populations of cytologically identical cells will have different antigenic properties and that they have different life spans, one of a few days duration, the other of many years. The whole subject of the nature and function of the lymphocyte has become of immense importance and much research is at present under way on these topics.

Lymphocytes are often found in the tissues as well as in the circulation. As the numbers circulating vary relatively little and as large numbers are constantly being produced, a circulation of these cells between blood and tissues occurs through blood, lymph, lymph nodes and spleen.

(*b*) **Monocytes** (or large mononuclear cells) (3 to 8 per cent of the total number) Fig. 4.2.

The typical monocyte is 9 μm. to 12 μm. in diameter when fresh, but reaching to 20 μm. in a dry film when stretched out flat. The nucleus does not stain so deeply as that of the lymphocyte: it is usually ovoid or indented to a varying degree (sometimes very deeply), and placed excentrically. The chromatin has a characteristic tangled skein appearance, often with thickenings where the threads cross. The cytoplasm is abundant and weakly basiphil, staining blue with Romanowsky stains; in stained films the monocyte is most easily distinguished from the large lymphocyte by the much greater amount of cytoplasm relative to the nuclear volume. It used to be held that living monocytes were characterized by the presence in their cytoplasm of a rosette of granules stainable with neutral red; it is now known that neutral red stains lysosomes and as these occur in other cell types, this staining reaction loses its diagnostic significance.

The monocyte is characterized by the presence of a well-developed Golgi apparatus, many vesicles of the endoplasmic reticulum, some arranged as flattened cisternae, and numerous ribosomes and mitochondria.

When cultured outside the body, monocytes may show an extraordinary range of form, depending upon the constitution of the bathing fluids. They may also come to resemble macrophages, both cytologically and in their phagocytic properties, when they leave the blood and enter into the tissues. Not all tissue macrophages, however, should be regarded as being derived from monocytes.

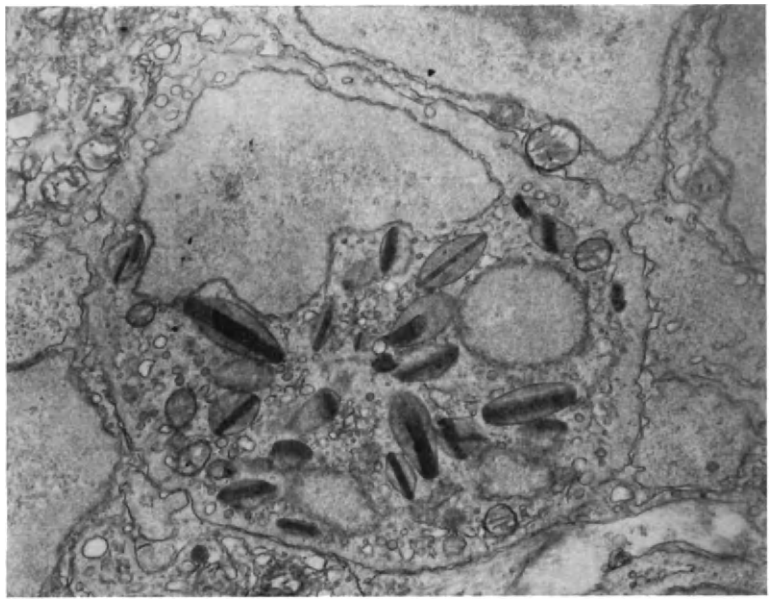
(2) Granular Leucocytes

The cytoplasm of these cells contains granules which, during life, are in a state of active movement: their size and staining reaction vary in the different groups of cells. In contradistinction to the non-granular forms, these leucocytes all possess a pleomorphic nucleus, subdivided by deep constrictions into a varying number of lobes, the number increasing with the age of the cell.

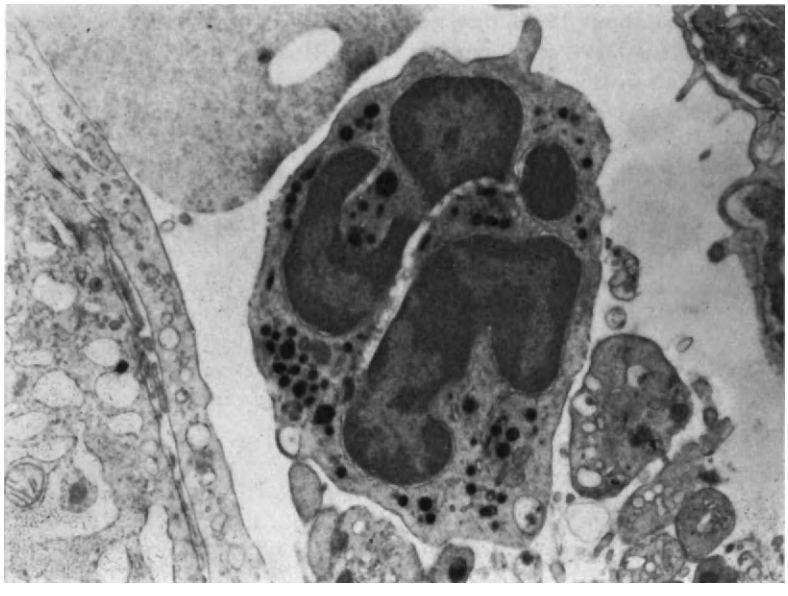
(*a*) **Neutrophils, or Neutrophil Polymorphonuclear Leucocytes** (65–75 per cent of the total number) Figs. 4.1, 4.2. The neutrophils are normally by far the most numerous of the leucocytes, and are easily recognized by their very fine granules and by their extremely lobulated nucleus. In humans the neutrophil granules stain a pale lilac colour with the Romanowsky stains. In fresh blood the neutrophils are from 6–8 μm. in diameter but in a dried smear they measure from 9–12 μm. in diameter. If they are examined alive by phase-contrast microscopy on a warm stage they are seen to be actively motile, moving in an amoeboid manner.

The nucleus consists of several rounded lobes connected by thin threads of chromatin, the whole being twisted and elongated (Figs. 4.1, 4.2, 4.5B). Some 3 per cent of neutrophils from a female show the sex chromatin attached to one of the nuclear lobes by a thin stalk forming the so-called "drumstick".

The electron microscope shows the neutrophils to have few mitochondria and vesicles of the endoplasmic reticulum and only sparse ribosomes, but to have many electron dense granules (Fig. 4.5B). These have now been equated with lyosomes by virtue of their content of



(A) 1 μm.



(B) 1 μm.

FIG. 4.5. (A) An electron micrograph of an eosinophil which has migrated out from the blood stream into lymphoid tissue. The nucleus is sectioned in four places and the characteristic crystalloid may be seen in the specific granules.

(B) An electron micrograph of a polymorphonuclear leucocyte in a capillary. Several of the lobes of the nucleus may be seen, together with the electron-dense specific granules.

acid phosphatase and other hydrolytic enzymes. The Golgi apparatus of the neutrophil is poorly defined. Recent studies suggest that a neutrophil has an average life in the blood stream of about 60 hours before being lost by passage into the tissues or through the epithelium of the gut.

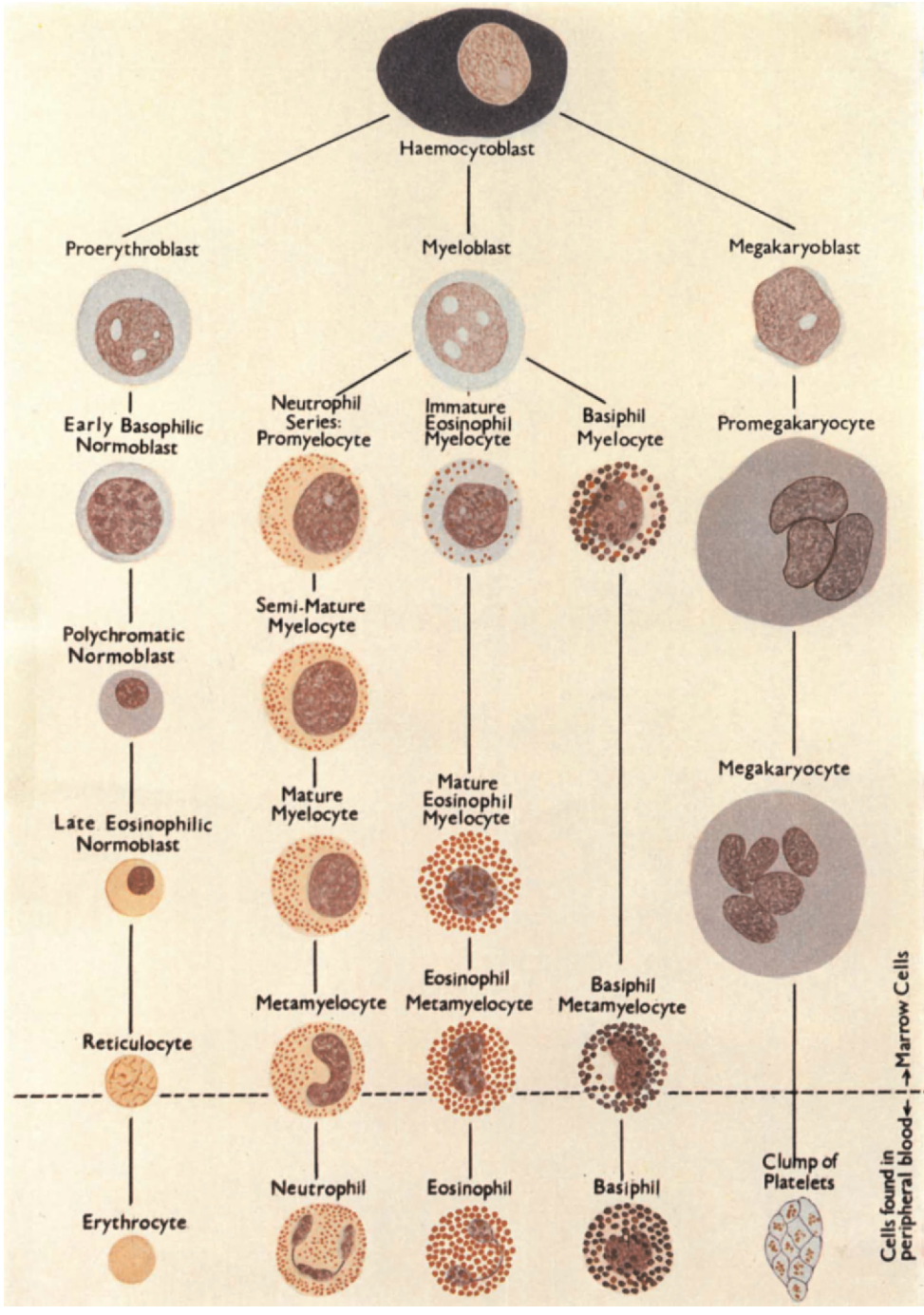
(*b*) **Eosinophils, or Eosinophil Polymorphonuclear Leucocytes** (2 to 5 per cent of the total number) Figs. 4.1, 4.2. These cells are spherical, about 9 μm. in diameter in the living state and about 12 μm. diameter in a dried film. The nucleus usually consists of 2 or 3 lobes connected by thin bridge of chromatin. The cytoplasm is slightly basic but this reaction is overshadowed by the prominent staining of the numerous large eosinophilic granules. In the living cell these granules appear highly refractile; they can be shown by cytochemical tests to contain lipid and many hydrolytic enzymes. This latter feature has resulted in most histologists classing the large eosinophil granules as lysosomes, a feature which they share with the specific granules of the neutrophil. Studies with the E/M show that the eosinophil has few mitochondria but that some endoplasmic reticulum can be distinguished among the specific granules. These have a very characteristic morphology, showing an internal crystalloid in the form of a band of parallel lamellae (Fig. 4.5A).

Eosinophils are thought to exist in the circulation for about 8–12 days.

(*c*) **Basiphils, or Basiphil Polymorphonuclear Leucocytes** (0·5 to 1 per cent of the total number) Fig. 4.2. In a dry smear their diameter is about 10 μm. The nucleus is generally S-shaped and difficult to see because of the numerous cytoplasmic granules. These are large, spherical, basiphil and metachromatic. As they are soluble in water they are often distorted in preparations stained with aqueous dye solutions. The basiphil is a cell which shows very active amoeboid movement. The E/M has shown that the granules are membrane-bound and have a highly ordered internal patterned structure which shows specific variation. The life of a basiphil is thought to be about 12–15 days. The basiphil is probably the same cell type as a mast cell, though it seems more appropriate to keep this latter name for cells with basiphil granules which are found in the connective tissue.

A summary of the characteristics of the various categories of leucocyte is given in the table below.

Cell type	Percentage of total number of leucocytes	Diameter in dry smear	Nucleus	Cytoplasmic Granules	Amoeboid Power	Phagocytic Power
Lymphocyte	20–25	7–10 μm.	Spherical nucleolus.	None	Not shown in blood, but potentially +.	—
Monocyte	3–8	14–20 μm.	Ovoid or indented. 1 or 2 nucleoli.	None	+ + +	Extravascularly + + +.
Granular leucocytes—						
(*a*) Neutrophil	65–75	9–12 μm.	Extremely polymorphic. No nucleolus.	Small, very numerous. Neutrophil.	+ + +	+ +
(*b*) Eosinophil	2–5	12–14 μm.	Usually 2 or 3 lobes. No nucleolus.	Large, spherical acidophil.	+ +	?
(*c*) Basiphil	0·5–1	10 μm.	Usually S-shaped. No nucleolus.	Large, spherical basiphil.	+	Probably —.
Erythrocyte		6·7–7·5μm.	None	None	—	—
Platelet		3 μm.	None	Small, basiphil.	—	—



Stages in the development of blood cells.

[*To face p.* 52.

Effect of External Factors

The leucocytes are not affected so rapidly as are the erythrocytes by external variations, and they can be kept alive for some time in a drop of blood. In hypotonic solutions they swell up: in hypertonic solutions they shrink.

Functions of Leucocytes

The leucocytes are cells which are comparatively inactive in the blood stream which is for them, as for the erythrocytes, a means of transport: their functional capabilities become more apparent as they wander in the surrounding connective tissues.

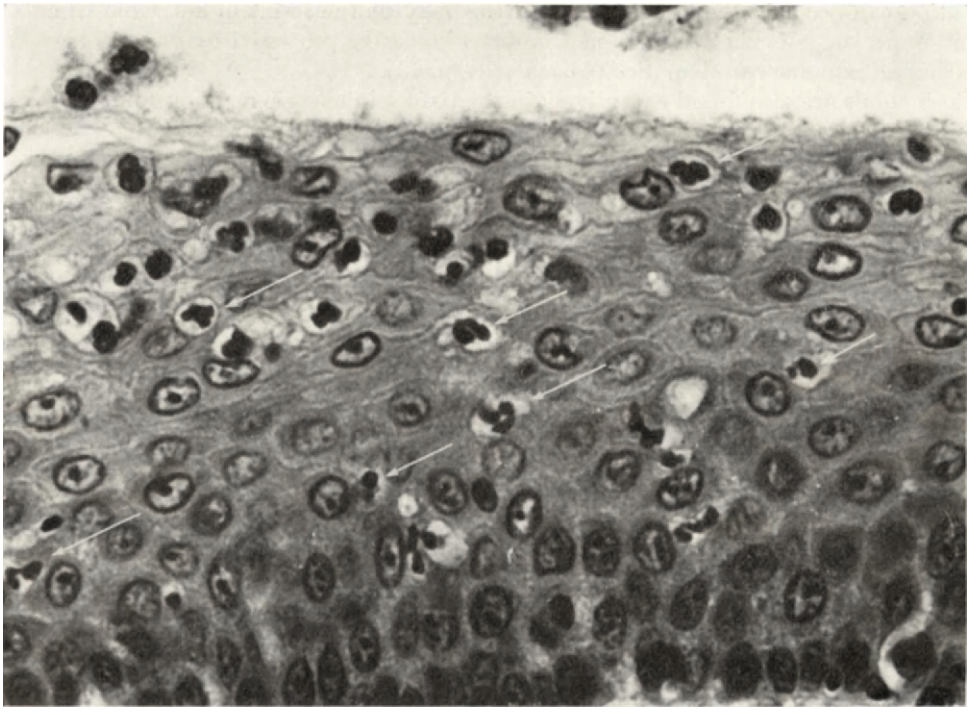


FIG. 4.6. A section through the epithelium covering the pharyngeal tonsil. Some local infection has occurred and many polymorphonuclear leucocytes (some of them indicated by arrows) are migrating through the epithelium. 20 μm.

(*a*) **Amoeboid Movement.** All leucocytes are capable of amoeboid movement, the neutrophil polymorphic cells being by far the most active and rapid. The monocytes are also very active, whereas the eosinophil and basiphil cells move very slowly: the lymphocytes display only little active motility. Because of this power there is a constant interchange of cells between the blood and the other connective tissues: in cases of need, such as in the inflammatory reaction to local injury (see p. 427), leucocytes migrate out of the blood vessels in large numbers into the surrounding tissue, the process being known as *diapedesis* (Fig. 4.6).

(*b*) **Phagocytosis.** Phagocytic activity in leucocytes is one of their most important functions The neutrophil polymorphonuclear leucocytes are the most active in this respect especially with regard to the ingestion of bacteria, against which they constitute one of the chief defences of the body. Their numbers increase enormously both in the blood and in the tissues when need arises. Phagocytosis of bacteria by neutrophils is accompanied by their digestion by the

enzymes of the leucocyte lysosomes and eventual death of the cell. Accumulations of dead neutrophils occur as *pus*. Monocytes, particularly when they have migrated out of the blood stream, also have marked phagocytic powers particularly for cell debris and foreign bodies. They play a vital role in removing damaged tissues and thus preparing the way for the regenerative processes of the body.

(*c*) **Immune Reactions.** Lymphocytes have been shown to be actively involved in immunological reactions, probably by acting as cellular agents able to recognize "foreign protein" and by acting as carriers for antibodies in the form of γ globulins. It is still not clear whether the lymphocyte itself can produce antibodies; small lymphocytes, suitably activated in tissue culture, will grow in size and accumulate much RNA in their cytoplasm. Morphologically these cells then become indistinguishable from plasma cells. It seems likely that this transformation can occur in the living body and this may be the result of antigenic stimulation. Recent work suggests that the "foreign proteins" must be processed by macrophages before initiating an immune reaction. (See plasma cells, p. 73).

Eosinophils are also found extensively in the tissues in allergic reactions, but their role in these conditions is still not clear, although it has been suggested that they are responsible for the phagocytosis and removal of the complex formed when foreign proteins are neutralized by an antibody.

(*d*) **Other Functions.** Enzymes can be demonstrated in some of the leucocytes and are probably associated with the destruction of phagocytosed particles. The neutrophil polymorphs contain proteolytic and oxidizing enzymes: a peroxidase reaction is also given by the eosinophil granules and sometimes by the monocytes, whereas lymphocytes contain a lipolytic enzyme.

Blood Platelets (Fig. 4.7)

In circulating blood the platelets are small, round or oval, bi-convex discs, of an average diameter of 3 μm. As soon as blood is shed, the platelets adhere to one another, rendering their enumeration very difficult: their number is generally given as 200 to 400,000 per c. mm. of blood and they are thought to circulate in the blood stream for about 3–5 days. When examining a preparation of fresh blood it is seen that the platelets quickly become granular and cluster together: they then break down into small granules and threads of fibrin appear radiating from them.

In smears the central part of the platelet is granular and basiphil (the *chromomere*), but there is no true nucleus. They have been shown to contain large amounts of phospholipid, ATP and glycolytic and oxidative enzymes, together with 5 hydroxytryptamine; this latter substance can cause vasoconstriction, so assisting in the clotting mechanism and in plugging small vascular damaged areas. Platelets are formed from the megakaryocytes in the bone marrow.

At least one of their functions is to assist in the clotting of blood, at first by forming a physical plug and later by helping in the formation of fibrin from the plasma fibrinogen by the liberation of thromboplastin (Prothrombin activator).

The Plasma

Histologically the plasma is structureless. It contains a large number of substances such as proteins (albumin, globulins and fibrinogen), traces of mineral ions, cations, anions and nutrients all dissolved in water which forms 90 per cent by volume of the plasma. Minute globules of fat (*chylomicrons*), 1–3 μm. in diameter and small particles (*haemoconia* or *blood dust*) of disintegrated corpuscles are also present. For its functions, which are intimately associated with those of the formed elements it contains, the student should consult a textbook of physiology.

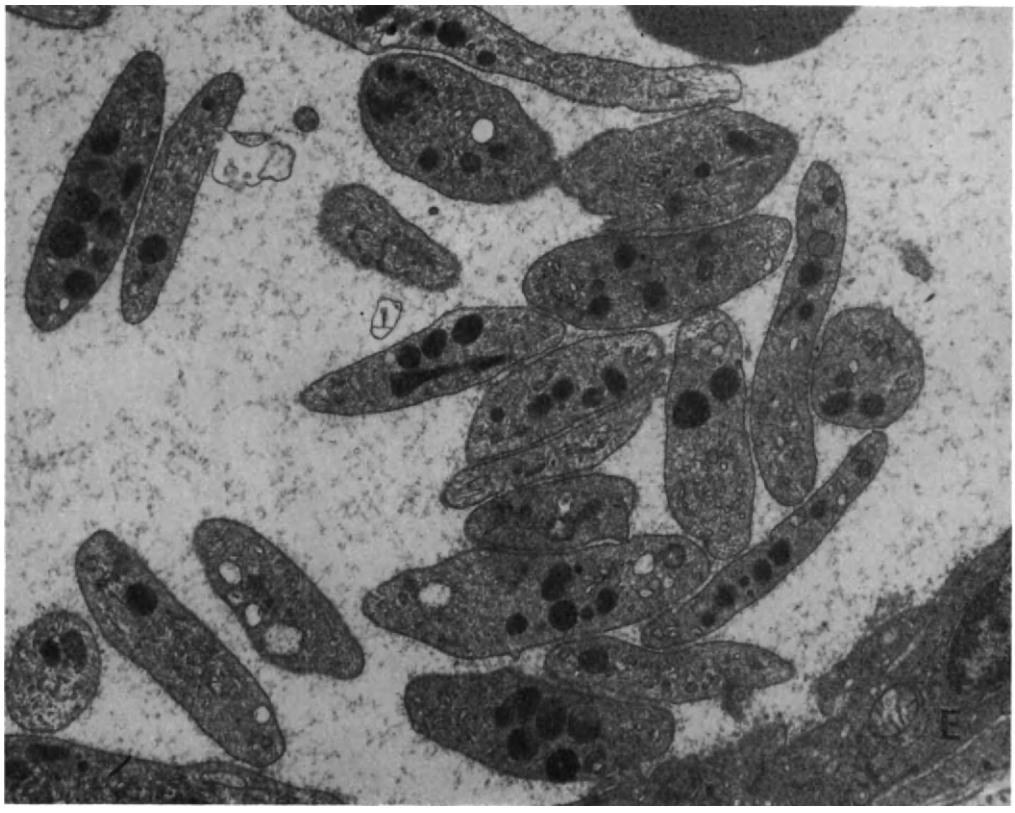


FIG. 4.7. An electron micrograph of a group of blood platelets in a capillary. Notice the vesicles and electron-dense granules in the platelets. The capillary endothelium (E) is at the bottom right of the micrograph. Electron micrograph by Dr P. Field.

1 μm.

LYMPH

Lymph is a colourless fluid formed by a process of filtration of colloid substances, salts and water from blood capillaries into the interstitial tissues and thence into a system of lymphatic vessels which eventually link up with the venous system via the thoracic duct and the right lymphatic duct. The lymph serves to return blood proteins from tissue fluids to the blood and maintains the turgor of the intercellular spaces.

The composition of lymph varies greatly in different parts of the body. The interstitial fluid is similar to blood plasma but contains less of the protein constituents. Lymph in the smallest vessels contains no cells, but these are added as the stream passes through the lymph nodes. The number of cells present varies enormously, but about 99 per cent are lymphocytes, usually of the small type, the remaining 1 per cent being made up of occasional erythrocytes, eosinophil leucocytes, or monocytes. During absorption the intestinal lymphatics contain large numbers of fat globules (*chylomicrons*), and the vessels were consequently called *lacteals* (p. 199).

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CHAPTER 5

DEVELOPMENT AND DESTRUCTION OF BLOOD CORPUSCLES. MARROW

I. DEVELOPMENT OF BLOOD CORPUSCLES (HAEMOPOIESIS)

The number of blood corpuscles does not increase by division in the blood stream; they are constantly being removed in large numbers, either when worn out or by migration into other tissues, and yet the relative and absolute numbers remain very constant. The corpuscles are continuously made outside the blood stream and added to it as required. The earliest stages in the development of the blood corpuscles are difficult to identify. There is general agreement as to the later stages of differentiation, but whereas some workers believe that all these cells are derived from one stem cell, others hold that there are two such stem cells. The former view is set out in the diagram on p. 60.

The haemopoietic tissue of the body is of two kinds:

(*a*) Lymphoid tissue, producing lymphocytes and monocytes.
(*b*) Myeloid tissue, producing granular leucocytes, erythrocytes and platelets.

A. Lymphoid Tissue

All types of lymphoid tissue* consist of two structures:

(1) A framework or stroma of reticular fibres and primitive reticular cells. The reticular cells give rise to the reticular fibres and almost certainly a significant number of the reticulum cells are actively phagocytic and may, therefore, be considered as fixed macrophages.

(2) Cells free in the meshes of the stroma: these are lymphocytes.

The simplest type of aggregation of lymphocytes is the solitary lymph nodule. In these bodies (which are frequent, for example, in a subepithelial position in the alimentary and respiratory tracts) the lymphocytes are closely packed. In the centre of the nodule is a zone of larger, less densely packed lymphoblast cells which, by mitosis, produce a peripheral zone of small lymphocytes. The central area of lymphoblasts is normally paler staining than the rest and is called the *germinal centre*. The phagocytic macrophages of the nodule act as a filter for the tissue fluids, and the small lymphocytes pass from the peripheral zone into the adjacent connective tissue.

The origin of the *monocytes* is a much debated question: four sources have been postulated:

(1) From primitive cells in the bone marrow.
(2) From the endothelium of blood vessels.
(3) From the fixed macrophages in various parts of the body, particularly the spleen.
(4) From the lymphocytes.

The most acceptable explanation of their origin would seem to be that monocytes develop primarily from undifferentiated, mesenchyme-like cells called *primitive stem cells*, situated in the bone marrow.

* See also p. 201.

B. Myeloid Tissue

In the adult human the myeloid tissue is normally confined to the red marrow. The primary function of myeloid tissue is to produce myeloid cellular elements for the circulation, but the marrow is also important on account of its content of phagocytic (reticulo-endothelial) cells and because it acts as a storage site for iron and for centres of immunologically-competent cells.

Structure of Marrow (Fig. 5.1)

Red marrow is found in the cavities of all bones for the first three or four years of life: as the subject grows older the red marrow recedes from the bones of the limbs, the change

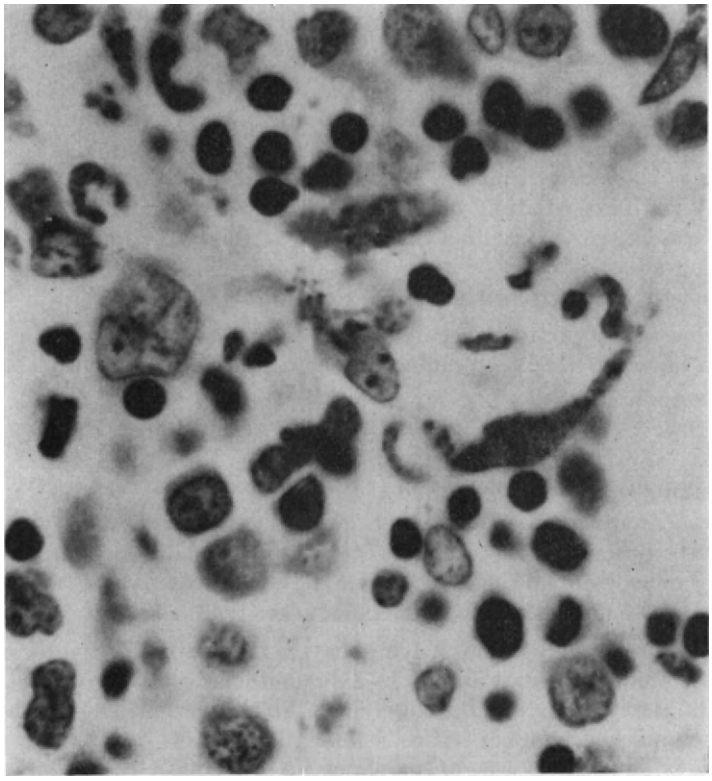


FIG. 5.1. Bone marrow prepared from an animal which has received an injection of an insoluble particulate dye. The elongated reticulo-endothelial cells of the marrow have accumulated the pigment granules within their cytoplasm. Many different stages of haemopoesis are visible; the prominent darkly-staining pycnotic nuclei are those of the late normoblasts. 10 μm.

beginning in the fingers and toes and spreading centripetally, leaving fatty yellow marrow in its track. This change begins at about the seventh year and is complete by the twenty-first. In each successive bone it recedes from the lower end to the upper, and leaves any bony epiphysis at the upper end before leaving the upper part of the diaphysis. The marrow in the ribs, sternum, skull and clavicles, vertebral bodies and pelvis remains red throughout life. The yellow marrow is chiefly fatty, and is not so richly supplied with blood as is the red marrow which is very vascular, having a rich circulation from the blood entering the bone via the artery of the nutrient foramen. There are no lymphatics in the marrow. If prolonged loss

of blood should occur the red marrow extends again to replace the yellow kind, which has no active haemopoietic power: in the infant there is no reserve space for extension of the red marrow, and after haemorrhage extra-medullary haemopoiesis may again take place.

Marrow, like lymphoid tissue, consists of two structures, stroma and cells.

(1) **Stroma.** The supporting stroma consists of reticular fibres and reticular cells, some of which have phagocytic powers (Fig. 5.1). Within this stroma the arteries and veins are united by numerous large, thin-walled sinusoids containing in their walls fixed reticulo-endothelial cells with indistinct boundaries which can round off to appear as free macrophages in the blood. [The sudden increase in the size of the vascular bed explains why metastases (e.g. cancer) so often settle there.]

(2) **Cells.** (*a*) *Adipose Cells.* These usually occur as isolated cells containing a single large droplet of triglyceride which displaces the cytoplasm and nucleus to form a thin peripheral rim around the fat droplet. (See p. 82.)

(*b*) *Mature blood corpuscles,* i.e. erythrocytes, granular leucocytes of the three types, and lymphocytes.

(*c*) *Haemocytoblasts* (Plate 1), the common stem cell of erythrocytes, granular leucocytes, and megakaryocytes. It is probably derived from the stem cells, and is large, about 15 μm. in diameter, with a large pale nucleus occupying a large proportion of the cell, and 3–8 nucleoli, and non-granular, deeply basiphilic cytoplasm.

(*d*) *Developing red corpuscles* (Plate 1 and Fig. 5.1) (normoblastic erythropoiesis). *Haemocytoblasts* multiply and develop into *pro-erythroblasts*: these in turn multiply by mitosis and give rise to basiphilic *early normoblasts*; these cells show many free ribosomes in their cytoplasm but as they mature they become smaller cells with less distinct nucleoli and less intense basiphilia of the cytoplasm. These cells again divide: the cytoplasm gradually becomes acidophil (eosinophilic) as accumulation of haemoglobin takes place and consequently at intermediate stages their staining reaction is both acidic and basic, giving the so-called "polychromatic" stage. The nucleus becomes darker and finally pycnotic (Fig. 5.1). The acidophilic *late normoblast* ultimately extrudes its nucleus, and the *reticulocyte* (see p. 48) results. Reticulocytes contain a tangled skein or reticulum in the cytoplasm demonstrable by supravital staining. This has been shown by the E/M to consist of remnants of endoplasmic reticulum. When the reticulum has disappeared the final product is the *erythrocyte*. Normal bone marrow contains about 30 per cent of erythroblasts* of all stages: in paraffin sections the majority of them show an eosinophilic cytoplasm.

(*e*) *Developing granular leucocytes. Myeloblasts* are also derived from haemocytoblasts. Granules develop in them and their nucleoli become less distinct, and the *myelocytes* result. (Early forms are pro-myelocytes, late forms meta-myelocytes.) In myelocytes the nucleus is spherical or slightly indented. They become differentiated into *neutrophil, eosinophil* and *basiphil* varieties by accumulation of specific granules in their cytoplasm: once this differentiation has occurred the cell cannot change its character any more. The neutrophil myelocytes are the most numerous, and the basiphil the scantiest. The myelocytes develop into the respective *polymorphonuclear leucocytes* with lobed nuclei.

(*f*) *Megakaryocytes* (Fig. 5.2). These are giant cells typically found in the adult in the bone marrow, but they also occur in the liver and spleen in the embryo; they may have a diameter of 40 μm. They are derived extra-vascularly from the haemocytoblasts by division of the nucleus and subsequent fusion of the daughter nuclei without any division of the cytoplasm: the fused nuclei often assume a horseshoe or ring arrangement.

The fully mature megakaryocytes produce the *platelets*. Behnke showed that by invagination of a large number of profiles of the external plasma membrane of the cell the demarcation membranes of the platelets were formed. These de-

* *Erythroblast* is a term that includes all nucleated red cells, normal or pathological.

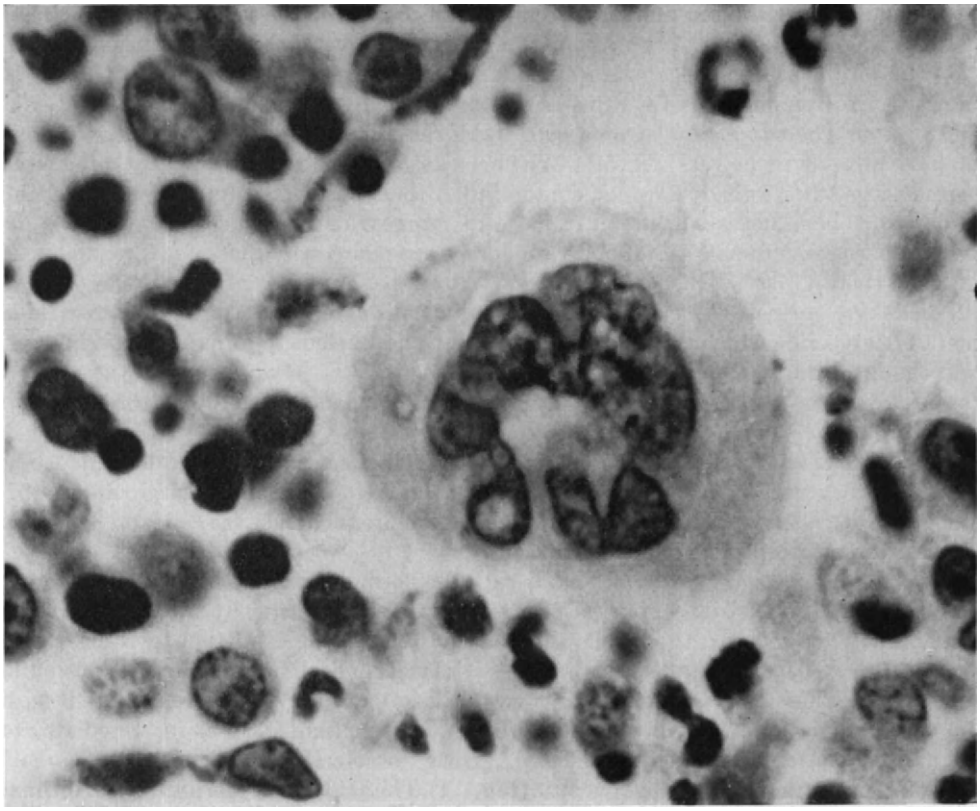


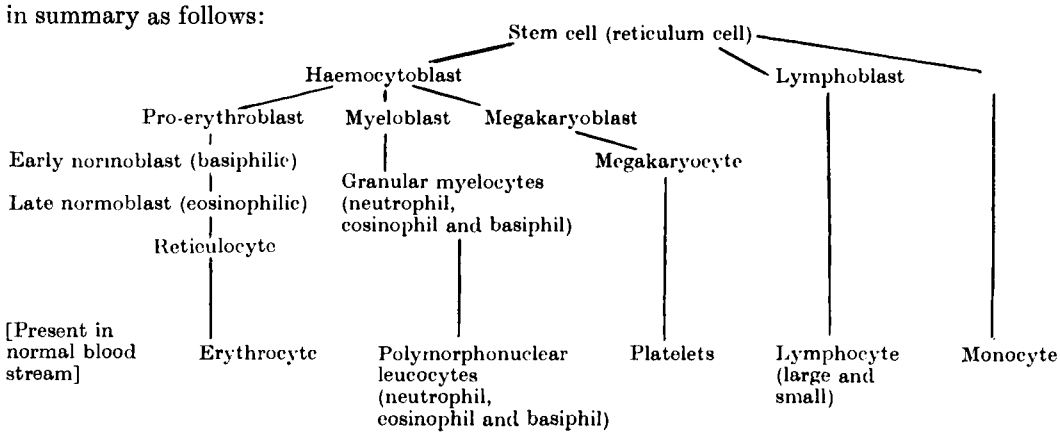
FIG. 5.2. A megakaryocyte in bone marrow; note the large size and the prominent lobed nucleus. 10 μm.

marcation membranes outlined the cytoplasm of future platelets which were then liberated by the extension of the demarcation membranes to fuse with each other.

(*g*) *Plasma cells.* These occur in bone marrow (to the extent of 1–3 per cent of the cell population) as isolated cells. (See p. 73.)

(*h*) *Foci of lymphatic tissue.* Small foci of lymphoid cells are often found in bone marrow; the amount varies from species to species. In man it also shows an increase with increasing age.

The relations of the various cells derived from the primitive reticular cell may be shown in summary as follows:



Entry of the Corpuscles into the Blood. When the corpuscles are ready for the circulation they enter the venous sinusoids of the marrow: the leucocytes migrate through the discontinuities which are known to be present in sinusoid walls. The exact mechanism of entrance of erythrocytes into the blood stream is not understood.

Embryonic Development of Blood

(1) **Extra-Embryonic Mesoderm.** The earliest formation of blood corpuscles is in the blood islands which appear from the 2nd week onwards in the extra-embryonic mesoderm covering the yolk sac and lining the chorion. The mesechyme cells proliferate actively to produce closely-packed cellular masses: these cells differentiate in two directions; the peripheral ones become flattened to give the primary endothelium of the earliest blood vessels, while the central ones become detached to float as primitive haemocytoblasts in the plasma. These primitive blood cells are rounded and their cytoplasm is basiphilic: they proliferate quickly, producing two new types of cells. Most of them acquire haemoglobin, becoming primitive erythroblasts, while the others remain as primitive haemocytoblasts. The primitive erythroblasts multiply in the haemopoietic sites, and in the foetal blood stream and develop into primitive normoblasts and eventually erythrocytes. Nucleated red cells begin to disappear from the circulating blood about the middle of foetal life. Later a few definitive erythroblasts appear; these are smaller than the so-called "primitive" cells, have a denser nucleus and form typical anucleate erythrocytes. Primitive leucocytes develop from the primitive mesoderm extra-vascularly and pass through the blood vessel walls into the blood stream.

(2) **Vascular Endothelium.** In the early stages of development the endothelial cells in certain blood vessels (e.g. caudal aorta) may proliferate and detach themselves as haemocytoblasts into the blood stream. This activity is also shown by the vascular endothelium in the liver, spleen and bone marrow in early embryonic development: later this power is lost as the cells are transformed into reticulo-endothelial lining cells.

(3) **Liver.** The liver develops as sheets of cells derived from the endoderm of the fore gut: these anastomose into a network in whose meshes are found large, thin-walled blood vessels. Between the vascular endothelium and the liver cells is mesenchyme from which differentiate macrophages and haemocytoblastic cells: these latter cells proliferate from the 6th week onwards to form large masses of erythroblasts and myelocytes. The corpuscles, when ready for circulation, pass through the thin walls of the venous sinuses, the vascular endothelium having been transformed into the lining reticulo-endothelial or Kupffer cells. In the latter half of gestation, when the erythroblasts gradually diminish in the circulating foetal blood, mature erythrocytes are also formed. The haemopoietic activity of the liver begins to decline about the fifth foetal month, and subsides before birth.

(4) **Bone Marrow.** By the end of the second foetal month the primitive marrow is formed from the mesenchyme that invades the cartilaginous forerunners of developing bone: as in the liver, primitive reticulo-endothelial and haemocytoblastic cells differentiate from the mesenchyme. By proliferation of the latter the typical myeloid and erythroid cells develop. After birth the crythrocytes are exclusively formed by the haemopoietic marrow, but in certain pathological conditions the liver, spleen and lymph glands may revert to haemopoiesis again.

(5) **Thymus.** This organ becomes an active lymphocytic centre at about 8 weeks and from then onwards functions as the most active embryonic site of lymphopoesis.

(6) **Spleen and Lymph Nodes.** The structure of the spleen and lymph nodes is described in detail later (p. 201). In the embryo the spleen is primarily an erythroblastic organ becoming important in this respect at about 3 months and declining in importance at about 5 months. The lymphoid tissue of the white pulp develops later and at the time of birth lymphopoesis is predominant in the spleen. The white pulp of the spleen is ordinary lymphoid tissue and provides lymphocytes and monocytes throughout life. The red pulp consists of stroma and free

cells, the latter including all the elements of circulating blood and free macrophages of reticular cell origin.

In all collections of lymphoid tissue in the embryo are found amoeboid cells (derived from the mesenchyme) which give rise to cells of reticulo-endothelial or haemocytoblastic type. Most of the latter proliferate into free lymphocytes, but granular myelocytes can also differentiate, and are commonly found in embryonic lymphoid tissue. Eosinophils are particularly abundant in the foetal thymus.

II. DESTRUCTION OF BLOOD CORPUSCLES

Effete red corpuscles are constantly being removed from the circulation. Some are retained in the spleen and destroyed by the phagocytic cells there: some are similarly removed by the Kupffer cells of the liver. The majority, however, probably become fragile and fragment in the blood stream, the debris being removed by the macrophagous cells of the reticulo-endothelial system. The haemoglobin of the erythrocytes is split into haematin and globin. Haematin is further broken down into iron (which is stored for reuse) and bilirubin which passes via the liver and the bile into the alimentary canal.

Leucocytes may degenerate in the circulating blood and be removed by phagocytic activity of cells of the reticulo-endothelial system. Many lymphocytes degenerate in the lymph nodes, and are removed in the same manner.

III. VARIATIONS IN THE BLOOD CELL PICTURE*

Any considerable haemorrhage results in loss of all the cellular elements of the blood: this is followed by a stimulation of haemopoiesis. The plasma volume is made up from the tissue fluids, this being the cause of the intense thirst after haemorrhage. The red marrow responds to the condition of depleted cells by active production of all elements, and if the need is urgent immature normoblasts, reticulocytes and myelocytes may be found temporarily in the circulating blood, and the red marrow may hypertrophy. The normal blood cell count is usually regained quickly. Degeneration of the red marrow affects the blood cell picture: this may occur as a result of insufficient nourishment, or of excessive strain, or of certain poisons or as a result of radiation damage. Extra-medullary myelopoiesis occasionally occurs in the spleen, liver and lymph nodes under pathological conditions.

Red Corpuscles

An *apparent increase* in the number of red corpuscles as estimated by the haemocytometer may occur simply by withdrawal of fluid from the blood (e.g. by sweating or diarrhoea): in this case there is no absolute increase in cells and the number returns to normal as fluid is again restored. At high altitudes there is an absolute increase in number, giving a count of 7,000,000 or more per c. mm., indicating a compensatory response to the lowered oxygen tension. A temporary increase in circulating red corpuscles occurs in conditions such as exercise and fear. This is due to the movement of red cells into the circulation from such situations as the sinuses of the spleen.

A *decrease* in the number is more common. It may be due to loss of blood, to deficient formation of new corpuscles, or to excessive destruction. *Anaemia* occurs if there is a deficiency in number of normal cells, or if the cells, although normal in number, contain too little haemoglobin. The cells may be deficient in number and yet each contain an amount of haemoglobin above the average (as in the large cells of *pernicious anaemia*), so that the colour index (see p. 46) is above 1. Anaemia is frequently associated with variations in size of the corpuscles (*anisocytosis*) or in their shape (*poikilocytosis*). There may be alteration in staining reaction

* For a detailed account of these variations the student may consult the book by Britton.

(*polychromasia*), and the appearance of basiphil granules (*punctate basiphilia*). Whenever haemopoiesis is actively increased immature forms (normoblasts and reticulocytes) may be found in large numbers in the circulating blood.

Megaloblastic erythropoiesis is normal in the early embryo but abnormal after birth. The cytoplasm becomes rich in haemoglobin earlier, and the nucleus remains immature longer than usual. This results, when accompanied by hyperplasia of the marrow (such as occurs in pernicious anaemia), in the appearance of megaloblasts and megalocytes in the blood stream.

White Blood Cells

There is an hourly rhythm of fluctuation in the numbers of polymorphonuclear leucocytes: a sudden great increase of deaths is followed by a marked increase in the number of healthy polymorphs, and the highest cell count may be double the lowest. The monocytes also show an hourly rhythm, with the highest count double the lowest: the lymphocytes have a half-hourly rhythm, and the highest count may be three times as great as the lowest. The average total number of white cells may be diminished (*leucopenia*) or increased (*leucocytosis*). In addition there may be a variation in the relative numbers of the different types of cells (as shown by a differential blood count) without alteration in the total numbers. In *leukaemia* there is a great increase of either granular leucocytes (*myeloid leukaemia*) or lymphoid leucocytes (*lymphatic leukaemia*). In myeloid leukaemia there is intense activity of the marrow, with an overflow into the blood, so that all stages from myeloblast to granular leucocyte are found circulating in large numbers: the spleen is enlarged due to extra-medullary myelopoiesis and also to deal with an increase in number of senescent leucocytes. Leukaemia is nearly always associated with anaemia.

Variations in the differential white cell count throw considerable light on the functions of the different types of leucocytes.

Neutrophilia (increase in number of neutrophil polymorphonuclear leucocytes). This occurs in any acute infection and in any inflammatory condition characterized by migration of these cells into the tissues. They are concerned in actively combating bacteria and toxins.

Eosinophilia (increase in number of eosinophil polymorphonuclear leucocytes). This occurs when there is much local migration of these cells into the tissues, and is found in conditions involving difficulty in respiration (e.g. asthma), in many types of dermatitis, and in infection with certain intestinal parasites. The eosinophils may be concerned with reactions to protein antigens and allergic phenomena.

Basiphilia (increase in number of basiphil polymorphonuclear leucocytes). Variations in the numbers of these cells are infrequent and their significance obscure.

Lymphocytosis (increase in number of lymphocytes (usually relative only)). The number of lymphocytes is normally higher in children than in adults. They migrate into the tissues in inflammatory reactions (see p. 427), at first in small numbers with the polymorphonuclear cells and then continuing in large numbers for a long time: they represent the reaction to a chronic inflammatory condition. As a rule small lymphocytes predominate in chronic conditions and large monocytes in more acute infections. The increase of these cells in the tissues is not necessarily accompanied by an increase in the blood. In lymphatic leukaemia there is an enlargement of all lymphoid elements in the body (such as lymph nodes, spleen, tonsils), and the production of lymphocytes is increased sometimes to as much as 95 per cent of the leucocytes present.

The Arneth Count

The degree of lobation of the nucleus of the polymorphonuclear leucocytes is of clinical interest. Arneth divided these cells into five groups; in Group I the nucleus is uni-lobed, in Group II bi-lobed, and so on (Fig. 5.3): the number of lobes, as shown from experimental

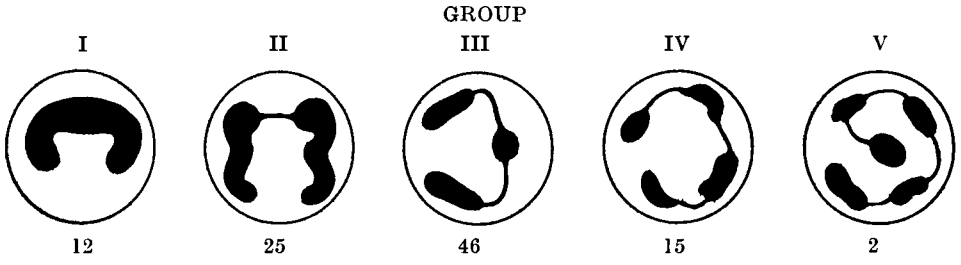


FIG. 5.3. Polymorphs which constitute the different groups in the Arneth count.

evidence, increases with the age of the cell. A white cell count is made and the average percentage number of cells in each group determined.

A relative increase in Groups I and II (shift to the left) indicates reaction in the bone marrow which releases additional polymorphs. This occurs in infection and toxaemia and also after haemorrhage: when the sum of Groups I and II is greater than 45 the count is abnormal. A relative increase in Groups IV and V (shift to the right) indicates that polymorphs are disappearing and that the bone marrow is unable to release more or produce replacements: this occurs in pernicious anaemia.

References for further detailed study:

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C. J. C. Britton (Editor), "Disorders of the Blood", 10th ed. Churchill (1969).
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CHAPTER 6

THE CONNECTIVE TISSUES

The connective tissues of the body possess two characteristics in common:

(1) They are all developed from the embryonic mesenchyme, which is itself derived from the primitive mesoderm of the germinal disc.

(2) They all possess a relatively large amount of intercellular matrix and fibres of various types, so that the cells appear isolated from their neighbours: the cells are as a rule relatively less important than the intercellular matrix and fibres. The matrix is permeated by tissue fluid which resembles blood plasma but has less protein, usually more albumin and a variable content of salts and glucose.

Transformation of both cells and intercellular matrix leads to the differentiation of the four chief types of connective tissue found in the adult:

(1) Connective tissue proper
(2) Cartilage
(3) Bone

} in which the intercellular matrix varies from a soft jelly to a rigid mass, and usually contains fibres.

(4) Blood and lymph, in which the intercellular matrix is liquid.

In the adult these types are not always sharply differentiated from one another.

Connective tissue proper is of various kinds, but the intercellular matrix always contains fibres. Areolar tissue, which has a very widespread distribution, is the most typical kind, and can be regarded as the general form from which the other varieties are specialized. These other kinds include elastic tissue (p. 75), white fibrous tissue and fibrous membranes (p. 75), mucous connective tissue (p. 77), reticular tissue (p. 77); adipose tissue is sometimes regarded as a variety of connective tissue but recent studies, especially by Wasserman, suggest that it should be regarded as distinct from connective tissue (see Chapter 7). In addition there remain to be considered the connective tissue of the central nervous system (p. 166) and the so-called reticulo-endothelial system (p. 78). For convenience, the serous membranes lining the body cavities will also be considered in this chapter.

A. Areolar Tissue (Loose Connective Tissue) (Figs. 6.1, 6.2)

Areolar tissue is the loose, irregular connective tissue that connects the skin to the underlying structures and fills any unoccupied spaces between organs: it penetrates with the blood vessels and nerves into the various tissues and organs, and is often stretched as a membrane between separated organs. It forms the delicate fascia of the intermuscular planes and of the palmar spaces, and constitutes the subaponeurotic layer of the scalp. In short, it connects parts but allows easy alteration in their relative positions. In the fresh condition it is soft and transparent and contains numerous potential cavities which pathologically may become filled with fluid (oedema): the presence of these spaces is responsible for the name (areola—a mesh). Connective tissue can show a marked reaction to various abnormal conditions, playing an important part in the reactions of inflammation (see p. 427).

Under the microscope it is seen to consist of innumerable single fibres and bundles of fibres running in various directions with a clear background (the intercellular matrix) containing a few cells: structurally the fibres are all extra-cellular.

(1) Intercellular Matrix

In a fresh spread preparation of areolar connective tissue the background appears to be transparent and homogeneous under the optical microscope. With the standard methods of

histological preparation no material can be stained, but evidence for the existence of the matrix was obtained several years ago. Most important was the fact that if fluid was injected into areolar tissue it did not disperse immediately but remained for some time as a discrete bleb. This was consistent with the interpretation that it was being physically contained by some viscous intercellular material. Again, if a fresh spread preparation was treated with silver nitrate, which was then reduced, the background areas appeared a dark brown in colour with clear areas which were presumably occupied by the cells of the connective tissue.

With the introduction of freeze-drying as a method of tissue preparation (which avoids the use of all fluids and reagents normally used) it proved possible to obtain strong metachromatic staining of the background matrix with dyes such as toluidine blue and also to obtain a positive PAS reaction (see p. 10). Both of these are indicative of the presence of protein polysaccharide complexes in the matrix. Recent biochemical studies have confirmed and amplified these observations. It is now known that the intercellular matrix contains several such complexes, differing in the degree and type of substitution of the sugar residue in their molecules. The predominating types in connective tissue are two very similar compounds—chondroitin 4-sulphate and chondroitin 6-sulphate—and hyaluronic acid, although others are found in certain more specialized tissues, e.g. the cornea of the eye. Chondroitin 4-sulphate has a molecule composed of a long chain formed of alternating saccharide units of 2-acetamido-2-deoxy 4-o-sulpho D-galactose and D-glucuronic acid, whilst hyaluronate has alternating units of 2-acetamido-2 deoxy D-glucose and D-glucuronic acid; in both cases there is a variable amount of protein present. The molecule of chondroitin sulphate, as its name implies, has a sulphate group in each repeating period (Fig. 6.3) whilst both types of compound have several carboxyl groups in their saccharide units. As a consequence of the presence of these ionized groups, the long chain molecules of these protein/polysaccharide complexes carry a large number of negative charges along their length and so they are termed "*polyanionic*". In the intercellular matrix a corresponding number of cations (usually Na^+)—the *counterions*—are present. The presence of polyanions in the matrix is responsible for some of their properties, especially those which are of importance in the control of water binding and in the control of the passage of various nutrients through the matrix to the cells and tissues.

Another feature of these mucoprotein complexes is the fact that their long molecules are thought to exist in a tangled configuration so that they occupy a relatively large amount of space known as a "*domain*". It has been calculated for example that a hyaluronate molecule would occupy a sphere about 400 nm. in diameter, whereas a molecule of the soluble form of the protein collagen occupies a cylinder 1·4 nm. × 280 nm. Three of the collagen molecules would equal one hyaluronate molecule in weight but the single hyaluronate molecule occupies 25,000 × as much space as the three collagen molecules together. The existence of these large domains confers upon the intercellular matrix its degree of resistance to the passage of other molecules through it, passage facility probably depending more upon the size and shape of a molecule rather than on its chemical nature. Again, as a consequence of the high degree of tangling of the hyaluronate molecules the intercellular matrix has a high viscosity. This is of great importance in connection with the mechanical functions of connective tissue and also as a means whereby foreign bodies such as micro-organisms may be contained in a limited environment until they can be dealt with by the body defence mechanisms. If the enzyme hyaluronidase is injected into the matrix then the rapid depolymerization of the hyaluronate results in a rapid drop in the viscosity of the matrix and injected material may then spread quickly throughout the matrix. It is this enzyme which is thought to be secreted by some invasive bacteria which explains their rapid spread throughout the tissues. Hyaluronidase was the active principle of the "spreading factor", extracted in the 1920's by Duran-Reynals from testicles; he showed that bleb formation could be prevented if fluid and spreading factor were injected together into the connective tissue.

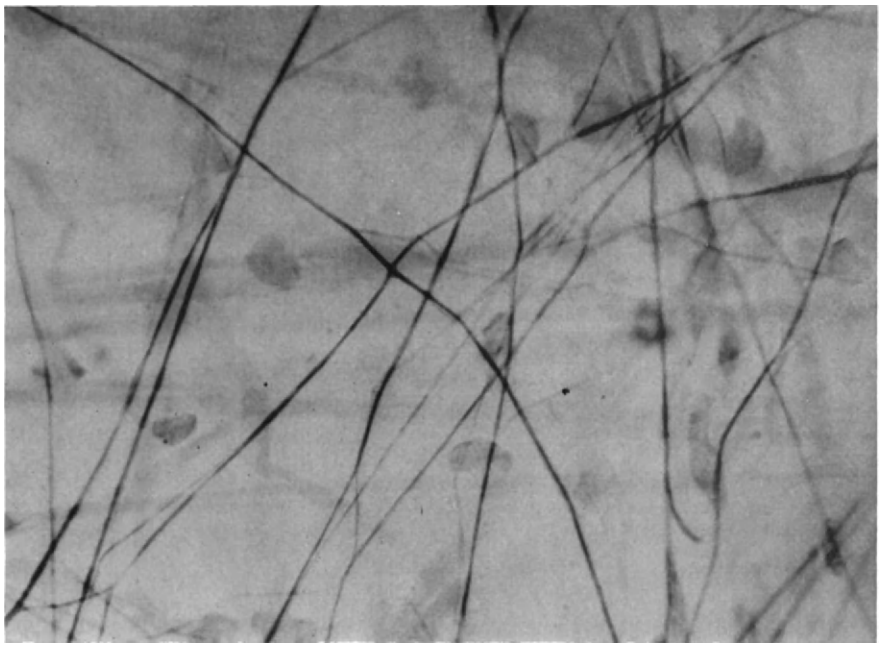

FIG. 6.1. A spread of areolar connective tissue, stained with orcein to show the elastic fibres. 15 μm.

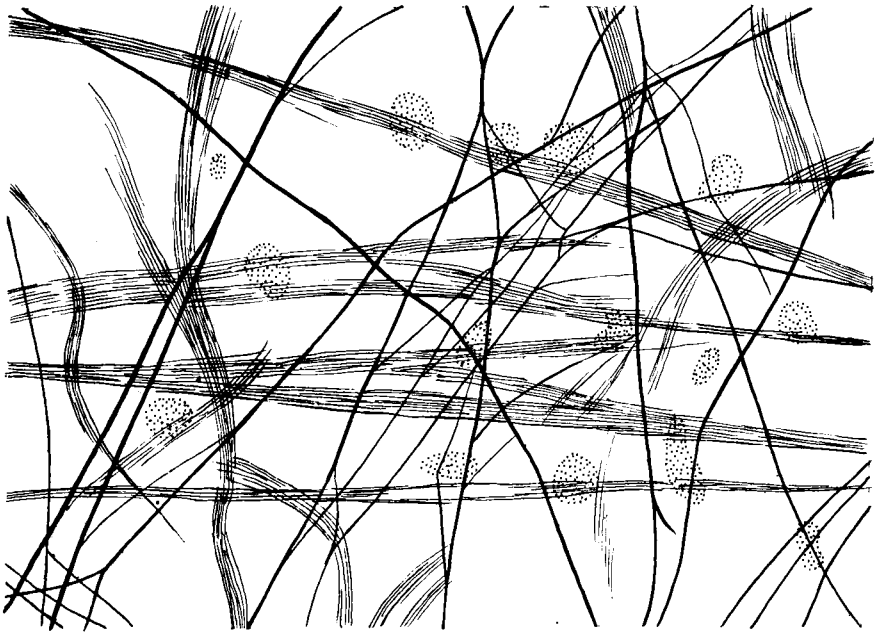

FIG. 6.2. A diagram of the same field as Fig. 6.1; note the single, branched elastic fibres, the less prominent bundles of collagen fibres and the nuclei (shown in stipple) which are principally those of fibroblasts.

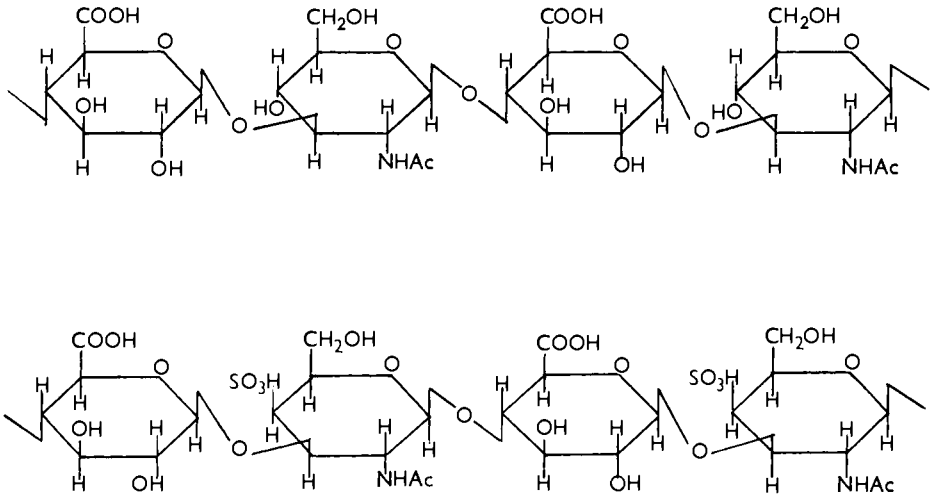

FIG. 6.3. The formulae of the basic repeating saccharide units of hyaluronic acid (above) and of chondroitin sulphate (below).

(2) Collagen Fibres

These appear under the optical microscope as straight or wavy bundles running in every direction. Each bundle consists of numerous fibrillae which do not branch, whereas the bundles of fibres often do. These fibres are soft, strong and flexible, but not elastic; they can resist a strain of several hundred kg/cm.^{2} without breaking and yet show an elongation of only a few per cent. They consist of collagen. This is a protein rich in glycine, proline and hydroxyproline

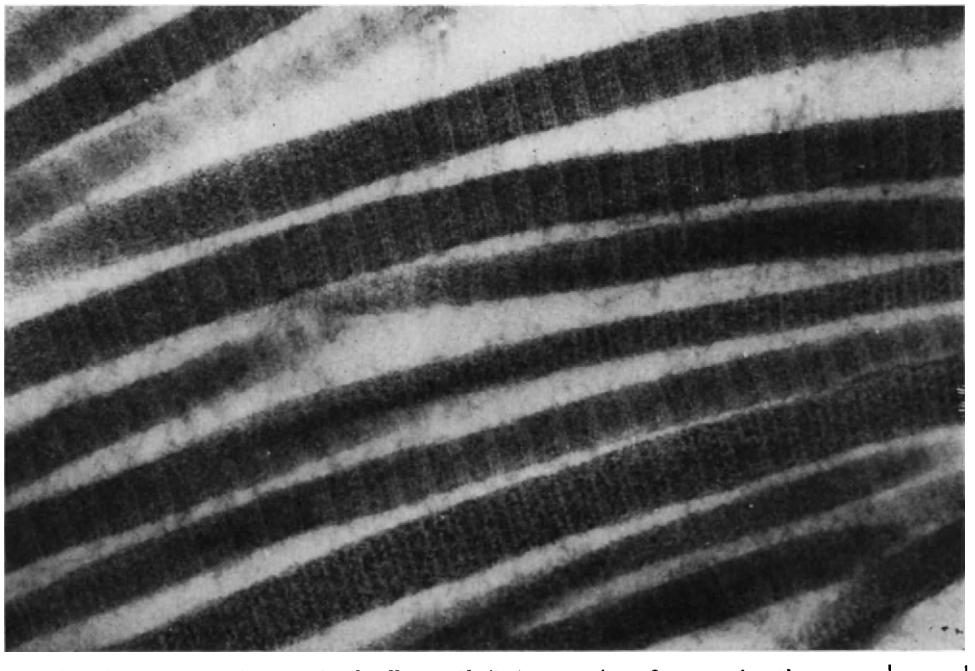

FIG. 6.4. An electron micrograph of collagen fibrils in a section of connective tissue. Note the marked periodicity of the fibrils.

and which yields gelatin on boiling. The fibres swell enormously in weak acid or alkali and are dissolved by pepsin in acid solution, but not by trypsin. Electron microscopy shows that the fibres can be further resolved into orientated fibrils 20–100 nm. in diameter, a fact which explains the fact that collagen fibres are birefringent when examined under polarized light. These fibrils possess cross striations with a marked periodicity of about 64 nm. (Fig. 6.4). When examined at high resolution with the E/M, several extra periods may be observed within the major periodicity. Recent work has showed that the individual molecules of collagen (known as tropocollagen) have a length of about 280 nm. Each consists of three amino acid helices intertwined to form a triple helical molecule. The striated collagen fibril which is

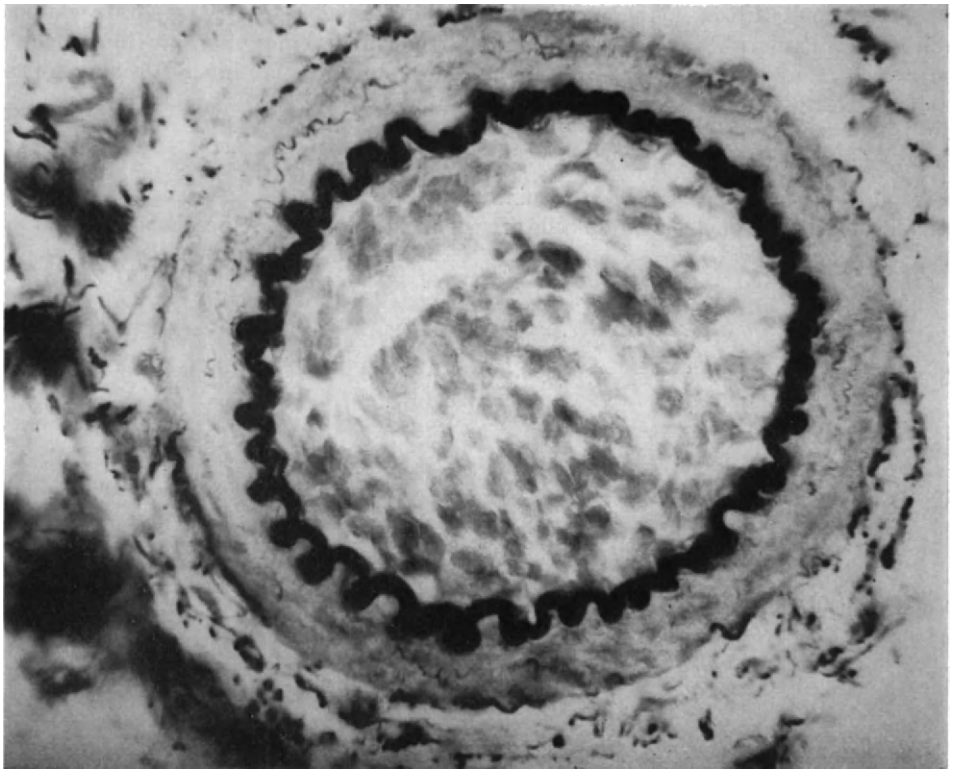

FIG. 6.5. The selective staining of the internal elastic lamina (shown dark) of a small muscular artery. Isolated elastic fibres may also be seen in the tunica media and adventitia of the vessel. The pronounced wavy appearance of the elastic lamina is due to the post mortem contraction of the vessel. 15 μm.

visualized in the E/M is formed by the staggered alignment of tropocollagen molecules. It is the overlapping of these molecules by about one quarter of their length which results in the formation of the major periodicity of the collagen fibril.

Very fine *reticular fibres* are also present in the matrix of areolar tissue. These fibres are probably of the same chemical nature as collagen fibres, but doubtless the molecules composing them are aggregated in a slightly different manner. They show a similar periodicity in the E/M. Reticular fibres tend to run in networks rather than aggregate into bundles and are important in the basal lamina and in lymphoid tissue (see Fig. 6.11). Reticular fibres may easily be distinguished in the optical microscope from collagen fibres by the fact that reticular fibres can be blackened by a suitable treatment with silver salts, whereas collagen assumes only a yellowish brown colour.

(3) Elastic Fibres

These are fine, straight, highly refractile fibres about 0·2–0·1 μm. in diameter which are composed of a protein known as *elastin*. The fibres branch and anastomose, run singly and not in bundles and are very elastic (Fig. 6.1). They break when the applied tensile strain is of the order of 20–30 kg/cm.^{2} but at their breaking point they will have extended to about 150 per cent of their original length. They show little birefringence in the natural state but become strongly birefringent on stretching. Elastic fibres may be stained selectively in sections by orcein or by resorcinfuchsin or by Verhoeff's stain (Fig. 6.5): they are partially dissolved by trypsin but treatment with the pancreatic enzyme *elastase* will digest them completely. Like collagen, elastin is rich in the amino acids glycine and proline; it has however a much higher content of valine. Under the E/M Ross has shown elastic fibres appear to possess two separate components; there is a central amorphous "core" surrounded by peripheral microfibrillar components. This region is rich in hydroxyl groups and in sulphur-containing amino acids and appears to develop before the central amorphous component.

(4) Cells

The following cell types can be recognized.

(*a*) **Fibroblasts (fibrocytes).** These cells are the most numerous in areolar tissue and may be

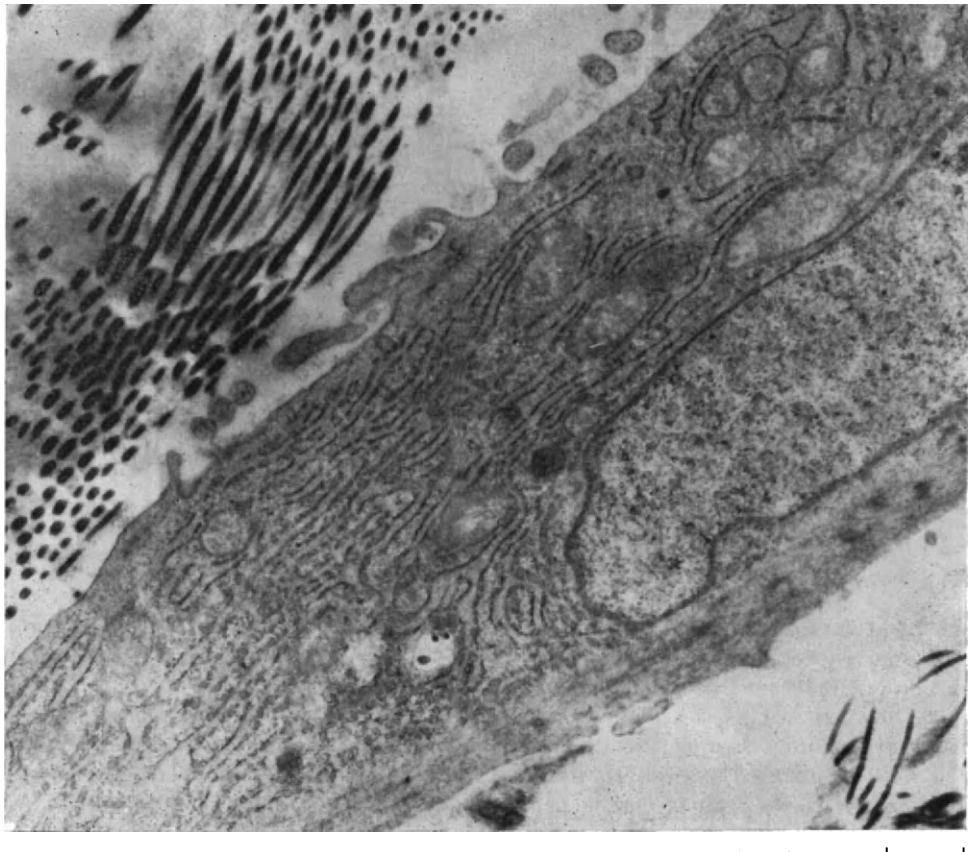

FIG. 6.6. An electron micrograph of a fibroblast in loose areolar connective tissue. Note the numerous profiles of rough-surfaced endoplasmic reticulum, characteristic of cells in active protein synthesis. The dark fibrils in the upper left hand corner of the micrograph are collagen but their periodicity is not apparent at this magnification. 1 μm.

thought of as forming a relatively stable population of fixed cells. They are flattened, with stellate processes, appearing fusiform in profile. The nucleus is ovoid with sparse chromatin and one or two nucleoli and the cytoplasm clear and very difficult to distinguish in either fresh or fixed, stained preparations. They are usually found closely apposed to the fibres and are concerned with their formation, hence the term fibroblast. These cells are found in all types of connective tissue proper. The manner in which collagen is formed from fibroblasts has been long debated. The favoured view, based largely on electron microscopy coupled with autoradiographic studies, is that precursor molecules are liberated from these cells and are then aggregated into fibrils either at or near their surfaces. The fibroblasts also appear to be able to secrete elastin.

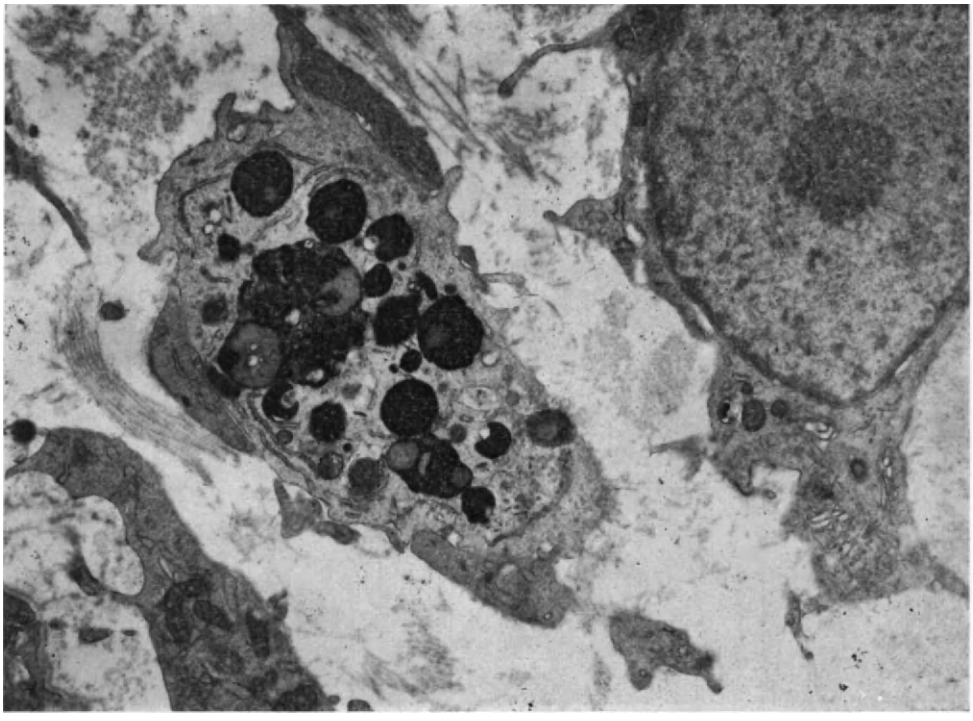

FIG. 6.7. An electron micrograph of a macrophage in loose areolar connective tissue. Large amounts of electron-dense cellular debris which have been phagocytosed by the cell are visible in its cytoplasm. The other cells in the micrograph are fibroblasts. 2 μm.

Normally the cells are resting and contain no inclusions: when in an active condition (e.g. during inflammation or in wound repair) they may contain many vacuoles. Although the branching processes of cells may come in contact with one another, there is no syncytium, each cell being independent. They can, under stimulation, undergo active proliferation, but they are specialized cells and do not give rise to the other types of free cells in the connective tissue. They play an important part in repair processes, in the manufacture of the mucoproteins in the intercellular matrix and in the formation of scar tissue (see p. 75). Their cytoplasm is rich in RNA and has a well developed granular endoplasmic reticulum (Fig. 6.6), especially when they are active in growth or repair processes. The quiescent fibroblast contains a small Golgi apparatus, which enlarges during activity.

(*b*) **Macrophages** (Histiocytes). These cells vary in number in different parts of the body, being most numerous in the areolar tissue of the very vascular areas, the mucous membrane of

the alimentary canal, the stroma of glands, and the serous membranes. The cells are usually round or pleomorphic; sometimes they are long and spindle-shaped and possess processes: the nucleus is small and stains more darkly than that of the fibroblast. The cytoplasm is granular and vacuolated and possesses large numbers of lysosomes. Macrophages possess the characteristic property of accumulating within their cytoplasm particulate matter which is stored within cytoplasm vacuoles. Organic material is then usually destroyed by the action of lysosomal enzymes but inorganic matter, e.g. carbon particles, may remain in their cytoplasm for long periods (Fig. 6.7). Under certain conditions (for example in inflammation) the macrophages in the connective tissue can become free, assume a rounded shape and transform into large amoeboid cells which migrate actively through the tissues. It is probable that many of the

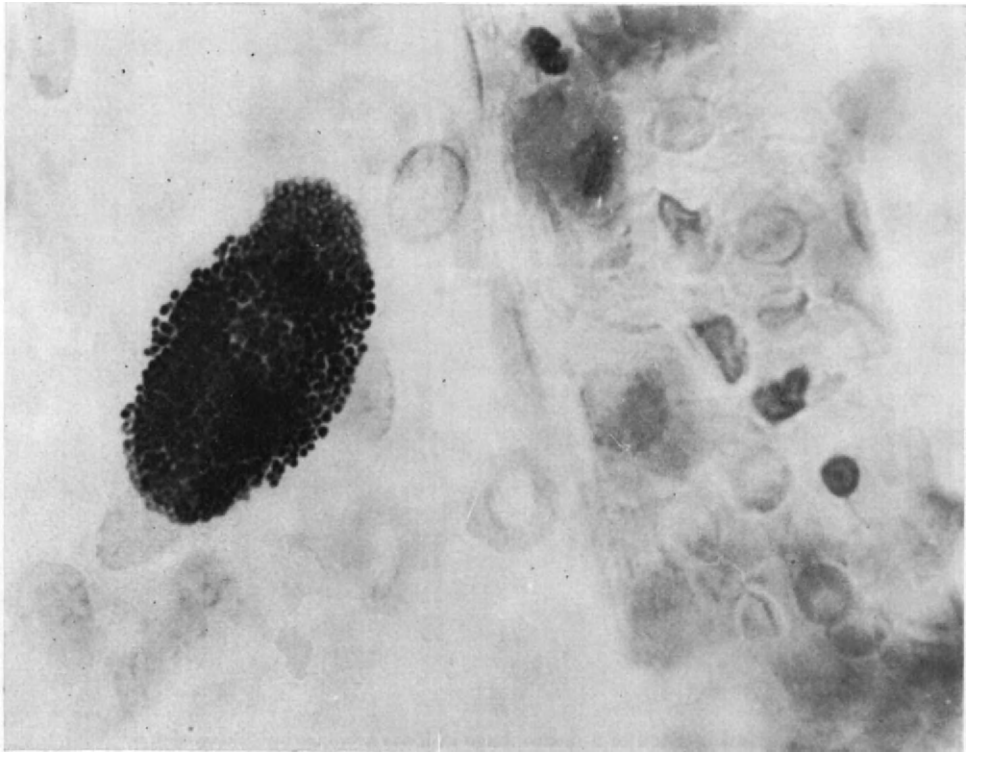

FIG. 6.8. A mast cell in the mesentery; this preparation has been stained with a basic dye to show the specific granules in this cell. Mast cells often appear in close relationship to small vessels, one of which may be seen on the right of the picture. 10 μm.

macrophages in the tissues are the monocytes of the blood which have migrated out from the blood vessels. Macrophages probably play an important role in the immunological defence mechanisms of the body by trapping, transporting and processing antigens which then affect the lymphoid responsive tissues. The presence of foreign bodies which are too large to be phagocytosed by a single cell may lead to the coalescence of macrophages to give rise to *multinucleated giant cells* (sometimes called foreign body giant cells) which surround and completely enclose the object.

(*c*) **Mast Cells** (Fig. 6.8). The number of these cells varies enormously in different animals and in different parts of the body. In man they are small, ovoid cells, often irregular in outline: in some animals they are large and may be elongated. The cytoplasm is filled with large granules (up to 1·6 μm. diameter in some species) which stain electively and metachromatically

with certain basic aniline dyes: thus methylene blue stains the nuclei pale blue and the granules dark purple. The granules contain a sulphated mucopolysaccharide which appears to be closely related to heparin. For this, and for other reasons, it has been suggested that the mast cells are concerned with the formation of the anti-coagulant heparin. More recently, it has been postulated that these cells might also be concerned in histamine production. These cells are probably related to the basiphil leucocytes of the blood.

(*d*) **Plasma Cells** (Fig. 6.9). These cells occur only occasionally in normal connective tissues; they are common in the serous membranes (particularly the omentum), in the alimentary mucous membrane and in lymphoid tissue. They are usually small and rounded, with a homogeneous basiphil cytoplasm. Adjacent to the nucleus is a pale area of cytoplasm which

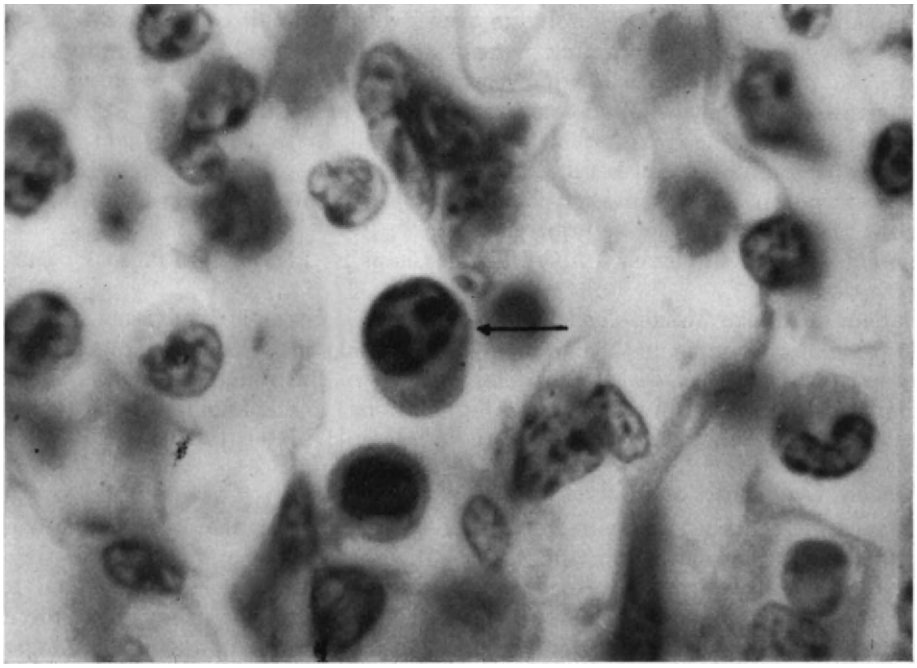

FIG. 6.9. Part of the red pulp of the spleen; the cell indicated by the arrow is a plasma cell. The characteristic clumping of the nuclear chromatin and the cytoplasmic basiphilia may be clearly seen. 10 μm.

the E/M has shown to contain the centrioles and a well-developed Golgi apparatus. The peripheral cytoplasm is full of flattened cisternae of the granular endoplasmic reticulum (ergastoplasm). The nucleus is small and excentric, and the coarse chromatin structure is arranged in radiating clumps adherent to the nuclear membrane: these stain deeply, giving a distinctive "cart-wheel" effect. Plasma cells may originate from undifferentiated mesenchyme-like cells (primitive reticular cells), but the majority of them may well originate from the transformation of lymphocyte-like cells in the regional lymph nodes in response to an antigenic challenge. The plasma cells have no demonstrable phagocytic activity but there is much experimental evidence in support of the view that these cells produce antibodies. Certainly, their cytoplasm with its extremely highly developed ergastoplasm (see Chapter 2) is of the type normally associated with a high level of protein production. They are increased in certain inflammatory conditions and in conditions where there is an antigenic challenge from a foreign protein.

(*e*) **Other Types of Cells.** (i) *Leucocytes*. Eosinophils are found in the interstitial connective tissue of the mammary gland, of the thymus and of the parathyroid, in the lung, in the omentum, in the appendix mucous membrane, and in the endometrium. They may increase in numbers in conditions such as allergic reactions, e.g. asthma and hay fever and in parasitic infections. The eosinophils are probably all derived from the blood stream. Their function is not known in these circumstances but may be concerned with the phagocytosis of antigen/antibody complexes. Small lymphocytes and monocytes also occur when there is a chronic, sub-acute infection, whereas neutrophils will be found in the tissues during the acute phase of an infection by micro-organisms.

(ii) *Undifferentiated Mesenchyme Cells*. Some of the original stellate mesenchyme cells remain undifferentiated in the loose connective tissue. They look like small fibroblasts and are usually arranged along the blood vessels. Under the influence of various stimuli these cells, unlike the already differentiated fibroblasts, can give rise to new cell types.

(iii) *Pigment Cells*. Cells containing pigment are found in man in the skin, in the choroid of the eye, and in the iris, such cells being very much more common in lower animals. They are small, elongated and branched, and contain granules of melanin (see Fig. 2.8).

(In some animals—e.g. frogs—the skin pigment cells are sensitive to light, and a redistribution of the granules produces a dark or a light skin effect. This can also be brought about in frogs by extracts of the pars intermedia of the pituitary: the response forms the melanophore test for the presence of this active principle.)

(iv) *Fat Cells*. (See also under Adipose Tissue, Chapter 7.) Droplets of fat may occur in any connective tissue cell.

Variations of Areolar Tissue. The relative amounts of fibres and of cells vary enormously in different parts. For example, in the dermal layer of the skin the collagen bundles are thick and arranged as a dense network, with delicate elastic fibres winding round the bundles, an arrangement similar to that found in scar tissue. All gradations may be found between typical loose connective tissue and the dense connective tissues described later (p. 75).

Blood Vessels, Lymphatics and Nerves of Connective Tissue

Areolar tissue contains very numerous blood vessels and lymphatics, many of which are passing to the adjacent epithelium and muscle. In subcutaneous and submucous tissues the lymphatics frequently form quite extensive plexuses. In fibrous membranes and tendons (p. 76) the blood supply is scanty, but superficial and deep lymphatic plexuses are present.

Many nerves are found in connective tissue, some passing on to epithelium and muscle and some ending within the tissue itself in special terminations, of which the Pacinian corpuscles are perhaps the most numerous (see p. 159).

Functions

(*a*) **Mechanical.** Connective tissues provide the supporting and connecting elements of the body; this includes the loose areolar tissue that provides a stroma for specialized structures, the tough fibrous coats and membranes, and the rigid masses of the cartilaginous and bony skeleton. The support which these tissues provide is largely dependant upon the close interrelationship between the fibres and the intercellular matrix. Each of these supplements the other exactly as the steel rods supplement the concrete matrix in the reinforced concrete used in building construction. If support is primarily needed at the cellular level, then reticular fibres tend to occur in a loose interwoven network around the cells, whereas if the support is needed for a tissue then the larger parallel bundles of collagen fibres tend to predominate. Again, if suppleness is required, as for example in the walls of a hollow viscus subject to periodic distension, then elastic fibres are found in large numbers. A good example is found in the walls of the alveoli of the lung where the elastic fibres help in the expulsion of air during expiration by their passive contraction following their stretching during inspiration.

(*b*) **Nutrition.** The intercellular matrix is largely involved in nutritional exchanges and in the maintenance of an adequate degree of hydration around the cells. Large amounts of water are bound by the intercellular matrix as a result of the polyelectrolyte nature of the mucopolysaccharide/protein complexes which form the bulk of this material. Again, as the molecules of the mucopolysaccharide/protein complexes are so large and occupy such large "domains" the porosity of the intercellular matrix is affected. The degree of resistance of the matrix to any molecule therefore depends largely on the size and shape of the molecule rather than upon its chemical identity.

Because of the possible occurrence of large numbers of adipose cells in the connective tissue this latter may become of great significance in regard to the storage of food reserve materials.

(*c*) **Defensive.** The cells that mobilize to defend the body against bacteria and other foreign bodies are connective tissue cells, whether blood leucocytes or macrophages: these cells are amoeboid and may exchange between the various phases of the connective tissue. The diffuse nature of the macromolecules in the intercellular matrix referred to above and the gelatinous consistency which they impart to it probably play a vital role as a mechanical barrier to the spread of micro-organisms which have penetrated into the body. This isolation helps contain them in a restricted situation until the cellular defence mechanisms of the body can be mobilized to deal with the infection.

(*d*) **Injury repair.** The fibroblasts which are found in all types of connective tissue are most important in the response to physical damage. They produce collagen fibres which serve to repair injury either in connective tissue proper or in other tissues which normally show little capacity to regenerate. Such a proliferation of fibres forms what is known as "scar tissue".

B. Dense Connective Tissues

(1) Elastic Tissue

Connective tissue in which the elastic fibres predominate is called elastic tissue; it often has a pronounced yellow colour when examined with the naked eye. It is found in those places where strength is required combined with the ability to be stretched and to return to its original form. It allows of structures being drawn apart and then aids in restoring and maintaining their relative positions at rest without itself being thrown into folds. The fibres vary greatly in size and tend to run in all directions, although they may be ordered in parallel bundles (as in the ligamentum nuchae). Often, as in the walls of hollow viscera where restoration of a resting configuration is required, the elastic tissue forms a fenestrated sheet (as in the internal elastic lamina of a small artery). It is often the case that where the overstretching of elastic structures is a possibility with the chance of rupture, white collagenous fibres are also present to limit the extension.

Chief Distribution

Ligamenta flava (between the laminae of adjacent vertebrae).
Ligamentum nuchae of large quadrupeds.
The deep layer of the superficial fascia of the lower abdomen (Scarpa's fascia).
True vocal cords.
Internal elastic lamina of muscular arteries.

(2) White Fibrous Tissue

White fibrous tissue consists almost entirely of collagen fibres running in bundles in definite directions: between the fibres are fibroblasts, and between the bundles of fibres is ordinary areolar tissue carrying blood vessels, nerves and lymphatic vessels, although these

are all sparse and hence regeneration after injury is often rather slow. Such tissue is found whenever great strength is required, combined with flexibility and resistance to a stretching force.

(*a*) *Tendons and Ligaments.* In tendons the collagen bundles (Fig. 6.10) run in parallel rows; between the bundles are found the parent tendon cells, which are fibroblasts that appear stellate in cross sections through having been squeezed between the fibre bundles. The whole tendon is surrounded by a capsule of coarse, fatty connective tissue, or by a fatty synovial sheath.

Ligaments, with the exception of the elastic ligaments, have the same structure as tendons, but the arrangement is not quite so regular.

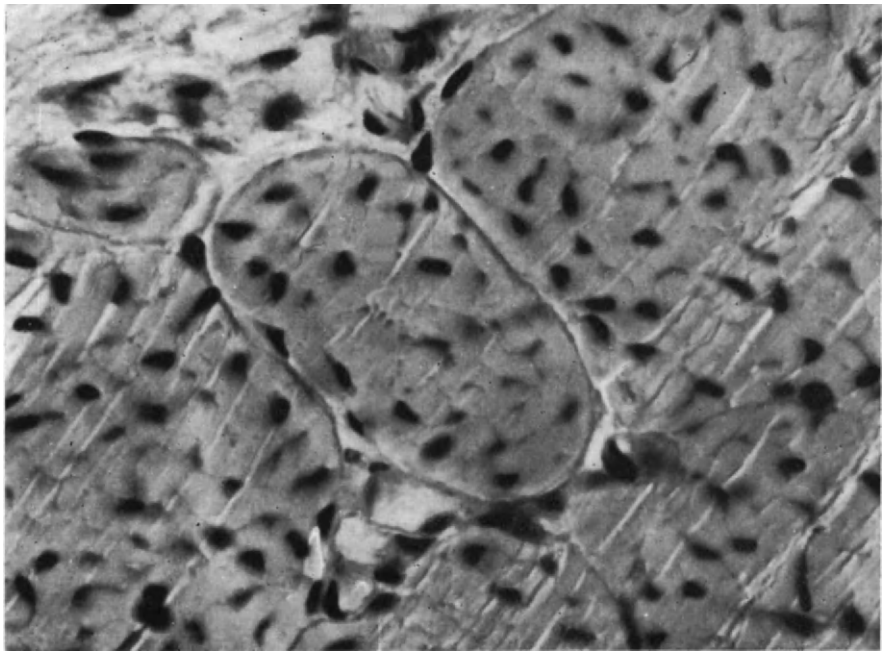

FIG. 6.10. A transverse section of part of a tendon. The nuclei of the tendon cells may be seen, but the individual collagen fibres in the tendon bundles are not visible. 10 μm.

(*b*) *Fibrous Membranes.* Such membranes occur in various parts of the body, and in addition certain organs are ensheathed in membranes of a fibrous nature. Here the collagenous fibres more usually have an interlacing arrangement so that they are better adapted to withstand tensions exerted from different directions.

(i) Some fasciae (e.g. fascia lata), aponeuroses, central tendon of diaphragm. The collagen bundles are arranged in shining white sheets of parallel fibres with fibroblasts between, as in tendons: the sheets run in various directions and fibres pass from one sheet to another. Fine networks of elastic fibres are found between the sheets.

(ii) Periosteum, perichondrium, dura mater, sclera of eye, fibrous pericardium, organ capsules. The tissue is opaque and white, the layers of collagen fibres run in every direction and the cells are usually fusiform or irregular. Such membranes blend without sharp distinction with the surrounding loose connective tissue.

(iii) Cornea. The tissue is quite transparent. For its structure, see p. 396.

C. Specialized Connective Tissues

(1) **Reticular Tissue** (Fig. 6.11). In certain places are found networks of fine connective tissue fibres. The fibres branch and anastomose and can be impregnated with silver by a Bielschowsky technique: they are regarded by most workers as immature collagen fibres and the difference in staining of these fibres and of collagenous fibres is due probably to the relative sizes of the fibres (see p. 69). The network of reticular (argyrophil) fibres supports the cells found in its meshes, and can be demonstrated easily as the framework of lymphoid nodules, of the marrow and of the spleen pulp: such a network is also present in many glands. When infiltrated with lymphocytes the underlying reticulum may be largely obscured: the reticular tissue and the lymphocytes together constitute lymphoid tissue (p. 201). In the blood-forming

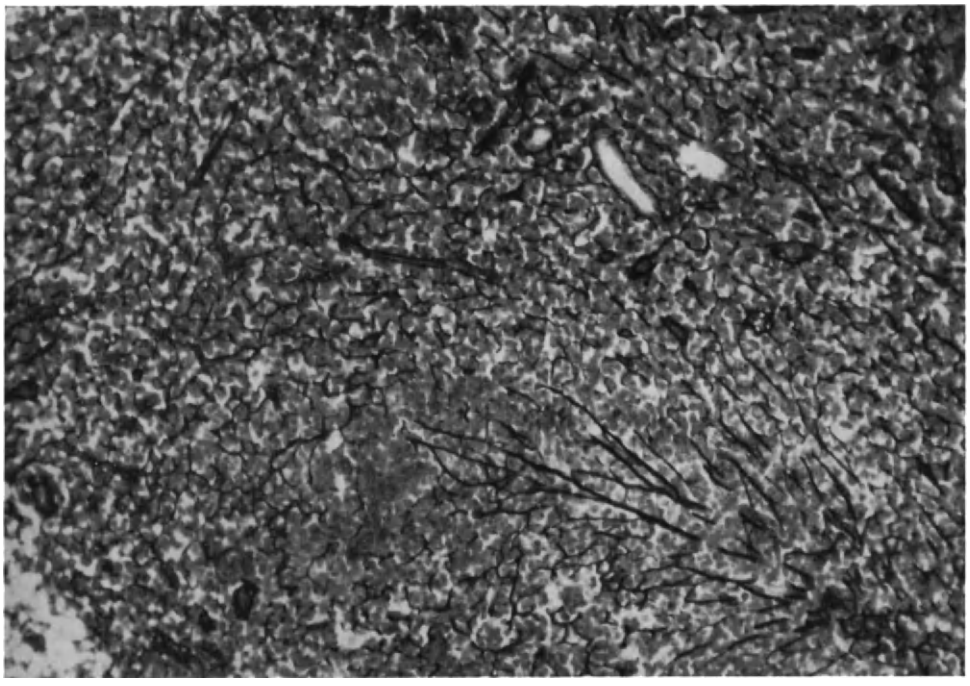

FIG. 6.11. Reticular fibres (in a section of a lymph node), rendered visible by impregnating them with silver. The cells in between the fibrous network are lymphoblasts and lymphocytes. 40 μm.

tissues (marrow, spleen and lymph nodes) the network of fibres is closely associated with the cells of the reticulo-endothelial system (see p. 78). Reticulo-endothelial cells are morphologically very similar to embryonic connective tissue cells or mesenchyme (see p. 74). In some areas, however, the fibre network seems to lack cells, so that there is a network of reticular fibres independent of the network of cytoplasmic processes of the reticulo-endothelial cells.

(2) **Mucous Connective Tissue.** This type of connective tissue occurs only during development and is not normally present in the adult human, although examples occur in adult animals of other species (e.g. the hypodermis of the sexual skin of some monkeys; the cock's comb). The best known example of mucous connective tissue in the human is the jelly-like matrix of the umbilical cord (Wharton's jelly). Its cells are stellate fibroblasts with long processes which often come into contact with those of adjacent cells: the matrix in which they lie is rich in hyaluronic acid (see p. 66). Some fine collagen fibres are present, together with a few wandering rounded cells, but elastic fibres are absent.

D. Macrophages and the Reticulo-endothelial System (see p. 71 and Fig. 6.7)

Scattered throughout the body are certain cells that are capable of ingesting foreign particulate matter such as artificially injected carbon particles, inhaled dust or bacteria. This may easily be demonstrated if a suspension of, say, indian ink or a dye called trypan blue is injected into the peritoneal cavity. The cells which ingest and concentrate such particles from their surroundings and either store or attempt to digest them belong to the system termed the "macrophage system" by Metchnikoff or the "reticulo-endothelial" (R.E.) system by Aschoff. The latter name is not very apt as the cells of the reticulo-endothelial system do not form an anatomical entity; many reticular cells are primarily concerned with fibre secretion rather than phagocytosis and true endothelial cells do not store or process any particulate matter which they may ingest. The name, however, has become accepted through long usage. The R.E. cells are also characterized by their possession of large numbers of cytoplasmic organelles called lysosomes; these fuse with the vacuoles containing any ingested material and if the material is of an organic nature, the lysosomal enzymes break it down. It is obvious that the marked phagocytic powers which characterize the cells forming this system are of great importance functionally. R.E. cells play a vital role in the local and general defence mechanisms of the body by sequestering invading micro-organisms and by clearing up cellular or other tissue debris which may result from any form of injury. The activity of the system is con stantly varying, being capable of stimulation by, for example, endotoxins liberated by bacteria and depressed by large doses of some drugs such as cortisone.

Chief Distribution of R.E. Cells

Macrophages of connective tissue.
Monocytes of the blood.
Primitive reticular cells and free macrophages of the spleen (see p. 211).
Macrophages lining the sinusoids of lymph nodes (see p. 203).
Macrophages lining the venous sinusoids of the spleen and bone marrow (see p. 58).
Kupffer cells in the walls of the intralobular sinusoids of the liver (see p. 323).
Macrophages among the lining cells of sinusoids in the suprarenal and anterior pituitary
Microglia of the nervous system (see p. 169).
Macrophages in the walls of lung alveoli that can mobilize and form "dust cells" (see p. 269).
Many authors would also include the neutrophils of the blood among the R.E. cells.

E. Serous Membranes

The closed body cavities are lined by serous membranes: mucous membranes line cavities which connect with the exterior. The serous membranes include the peritoneum, the pleura, the pericardium and the tunica vaginalis testis in the male. That part of the serous membrane lining the wall of the serous cavity is called the *parietal layer*: the membrane is reflected over the surface of the contained viscera as the *visceral layer*. As the membranes pass to the viscera, they may form folds (e.g. the mesentery) in which are found the blood vessels, lymph vessels and nerves destined for the organs: they may also form thin folds that hang freely in the cavity that they line (e.g. the omentum).

The inner surface of a serous membrane is composed of mesothelial cells which form a simple epithelium. Mesothelial cells resemble those of squamous or pavement epithelium (p. 33) but often they have less sinuous outlines. Marked microvilli and pinocytotic vesicles are shown by the E/M on the free border of these cells. If the membrane is forming a fold, then both free surfaces are covered by the mesothelium. This rests on a basal lamina that covers a thin layer of loose connective tissue containing a fine network of collagen and elastic fibres and the usual connective tissue cells: blood and lymph vessels lie only within the deepest layer.

The serous cavities contain fluid, the *serous exudate*. Under physiological conditions the amount of fluid is very small, but pathologically it may increase greatly. The cells floating in the fluid originate from the serous membrane: they include:

(1) Desquamated mesothelial cells—in inflammatory reactions these develop into actively phagocytic cells and possibly also fibroblasts.

(2) Small lymphocytes and occasionally eosinophil leucocytes which have migrated from the blood vessels.

(3) In pathological conditions serous exudates often contain large numbers of neutrophils from the blood stream; these cells are then often known as *polyblasts* (see p. 81).

The Peritoneum. This is the serous membrane lining the walls of the abdominal cavity as the *parietal peritoneum* and reflected over the contained viscera as the *visceral peritoneum* or serous coat.

The Mesentery. The mesentery consists of folds of the peritoneum attached to and surrounding the intestine. Both surfaces are covered with mesothelium, and between them is a network of collagen and elastic fibres, scattered fibroblasts, mast cells and resting macrophages, together with the blood vessels, lymphatics and nerves of the intestine. In addition there are usually many fat cells along the blood vessels and a large number of lymph nodes. Pacinian corpuscles are often present in large numbers: it is well known that the parietal peritoneum in particular is very sensitive to tension.

The Omentum. The omentum is similar in structure to the mesentery and consists of folds of the peritoneum attached to the greater curvature of the stomach and hanging freely into the peritoneal cavity. Parts of the omentum contain fatty tissue grouped round blood vessels, while other parts are extremely thin and contain only a few capillaries. In these thin areas there are many holes, so that a net-like structure is produced, the walls of the meshes consisting of bundles of collagen fibres covered on both sides with flat mesothelial cells. The thicker strands contain the small capillaries with their accompanying fibroblasts and other cells. The areas of the omentum that have no holes contain the same kinds of cells as loose connective tissue: these cells are numerous and active. Although the numbers of cells vary, the macrophages are particularly plentiful, as are also the small lymphocytes.

Physiological variations in the cell picture are due to the part played by the omentum in the absorption and detoxication of various endogenous metabolites. (See also physiological inflammation, p. 427.) Pathologically, similar substances of exogenous origin may penetrate into the peritoneal cavity; the omentum then responds by increased activity, with migration of granular leucocytes from the blood vessels and mobilization of macrophages.

Certain areas of the omentum are particularly rich in cells: these patches are known as "milky spots", from their opaque appearance, and are found near blood vessels. In these milky spots the macrophages multiply: they then migrate through the mesothelium and are found in the peritoneal fluid.

Development of Connective Tissue

The connective tissue develops from the diffuse mesenchyme of the embryo (Fig. 6.12). It consists at first of stellate cells, together with some polymorphous wandering cells all lying in a semi-fluid intercellular matrix. Most of the cells differentiate later into fibroblasts, but a few remain always undifferentiated (e.g. pericytes of capillaries, see p. 185). The primitive wandering cells increase in numbers and the majority of them give rise ultimately to cells of the macrophage group, namely, active and resting wandering cells of adult connective tissue, and to mast cells.

The fibres appear in the intercellular matrix, the collagen being laid down before the elastic fibres. The collagen fibres are at first very fine single threads arranged in networks: later, the fibrils are re-grouped into wavy bundles which increase in size and eventually pervade the ground substance. In the past there has been much debate about the formation of collagen

fibres. Recent investigations, using a variety of techniques including E/M autoradiography, have led to widespread acceptance of the belief that reticular and collagenous fibres are formed extracellularly by aggregation of tropocollagen molecules secreted by the fibroblast into the intercellular matrix. Some authors believe that the probable synthetic site of this tropocollagen is the ribosome of the fibroblast and that the product is passed via the cisternae of the endoplasmic reticulum to the vesicles of the Golgi apparatus, where the final stages of the synthetic process take place. Finally the Golgi vesicles carry the tropocollagen to the cell surface where it is discharged. An alternative hypothesis is that the tropocollagen is discharged directly from the cell by the endoplasmic reticulum cisternae and that the Golgi vesicles are concerned only with the secretion and liberation of the mucoprotein complex of the intercellular matrix

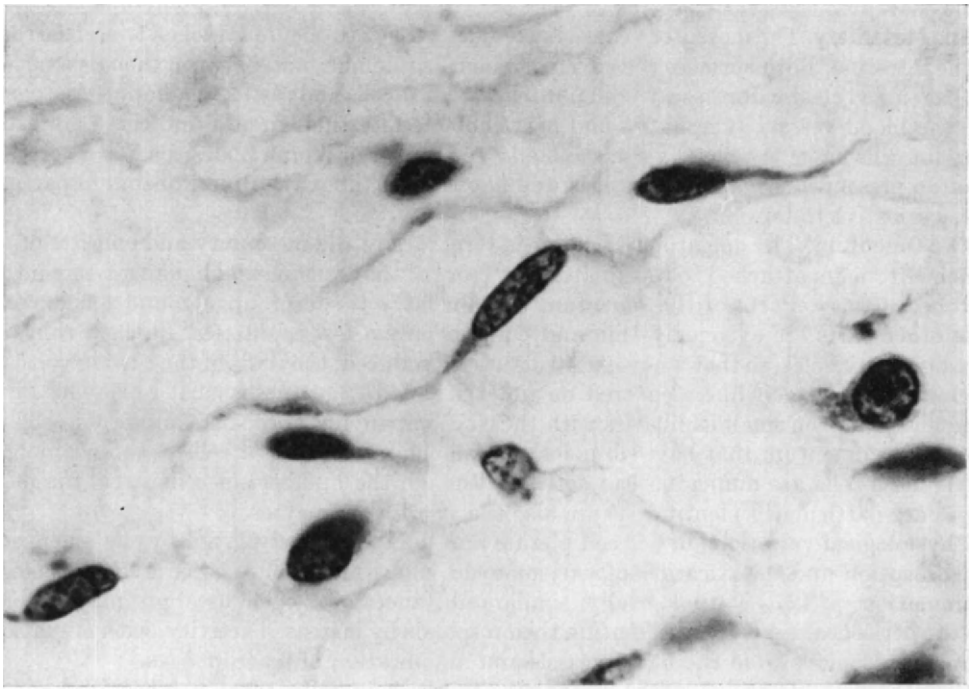

FIG. 6.12. An optical micrograph of embryonic mesenchyme. The stellate cells appear to possess prominent nuclei but little cytoplasm; the large amount of intercellular matrix is characteristic of this tissue. 10 μm.

itself. More experimental studies are required before it will be possible to decide which (if either) of these ideas represents the actual process of fibrogenesis.

The factors which are responsible for the extracellular aggregation of the tropocollagen molecules into fibrils are not yet understood. Some workers think that mechanical stresses in the developing tissue are primarily responsible for this whereas others think that the tropocollagen aggregates onto so-called "proto-fibrils" (not composed of tropocollagen) which are shed from the surface of the fibroblast itself. There is evidence from the study of wound healing in scurvy that vitamin C plays an important role in fibrogenesis. In the absence of this vitamin there is a deficient formation of collagen and the intercellular matrix is atypical. The origin of the intercellular matrix itself is a matter of much debate, and even more uncertainty. Fibroblasts are now considered responsible for its production.

Elastic fibres appear later in the embryo, their development being closely correlated with the functions of the parts concerned. Little is known about their formation, but probably a

precursor (analogous to tropocollagen) is secreted by mesenchymal cells which are most likely morphologically identical with fibroblasts.

Inter-relationships of Blood and Connective Tissue Cells

Under physiological conditions it is difficult to elucidate the relationships between the various cell types, but the links are made clearer by studying the reactions occurring in local inflammation in the body and in tissue cultures outside the body.

In inflammation of the connective tissue three cell types can be distinguished (see also p. 427).

(1) **Fibroblasts.** These proliferate, but keep their original form and are *not* transformed into wandering cells or leucocytes. They are concerned with collagen formation in the scar tissue.

(2) **Polymorphonuclear leucocytes.** These migrate out from the blood vessels, and, having done their work of phagocytosing micro-organisms, rapidly degenerate; if they are numerous they form pus.

(3) **Non-granular amoeboid mononuclear cells, of various forms, called polyblasts.** Some of these arise from the local macrophages, but many more are differentiated from lymphocytes and monocytes that have migrated out of the blood vessels. It is probable that these polyblasts become differentiated into plasma cells (p. 73) which secrete the antibodies.

In tissue culture the same type of cell changes occur. Fibroblasts proliferate rapidly but never become amoeboid, nor are they differentiated into another kind of cell. The monocytes of the blood can become changed into large amoeboid phagocytic cells and, as development proceeds, it has been claimed that these in turn become fibroblasts. Such experiments should be interpreted with caution since the transformation of *individual* cells has never, so far, been observed. Small, adult, lymphocytes do not develop into any other type of cell on culture.

Thus the fibroblast is to be regarded as a highly differentiated type of cell that does not give rise to other varieties. The macrophage and small lymphocyte-like type of cell, on the other hand, can produce the various kinds of cells that take part in defence reactions—the polyblasts, plasma cells, giant cells. The monocytes of the blood are typical macrophages.

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CHAPTER 7

ADIPOSE TISSUE

Adipose tissue (fatty tissue) is of widespread distribution in the body; it is found in the superficial fascia below the skin, in the axilla, bony orbit, mesenteries and omentum, around the kidneys and in the marrow cavities of long bones, to mention only a few of the more obvious locations. Until recently this tissue was regarded as an inert, passive packing material and a surplus food store. It was thought to be of little importance in the body and was classified as a variant of connective tissue in which the cells had accumulated triglyceride within their cell bodies. The current approach, however, is to attribute considerable significance to adipose tissue as an organ which, although so anatomically widespread, is so biochemically active and distinct that it should be regarded as a tissue in its own right.

It is possible on morphological grounds to distinguish two separate types of adipose tissue: white or yellow (sometimes called unilocular) fat and brown (or multilocular) fat. Of these the white adipose tissue comprises the bulk of the body's fat reserves, whilst brown adipose fat is much more restricted in its distribution.

A. White (Unilocular) Adipose Tissue

White adipose tissue is characteristically composed of large cells (often over 100 μm. in diameter) which are basically spherical but, because of their close packing, often assume a polyhedral appearance when seen in sections. The bulk of each cell is occupied by a single large lipid droplet which limits the cytoplasm and the nucleus to a small area at the circumference of the fat drop where they form a thin peripheral rim (Fig. 7.1). In an ordinary histological preparation the processing schedule has dissolved away the lipid droplet so that in stained preparations the adipose tissue has a characteristic "empty" appearance, often described as a "signet ring" appearance from the fancied resemblance of these cells with their displaced nuclei and peripheral cytoplasm to the outline of a signet ring. If frozen sections are prepared so that no organic solvents are needed in the processing, then the lipid may be coloured by certain dyes which dissolve preferentially in the lipid (Fig. 7.2). Chemical analysis of the lipid in adipose tissue has shown that in the human it consists principally of oleic acid with a smaller proportion of linoleic and palmitic acids. To a large extent this composition reflects the lipid composition of the diet. If corn oil (which is more than 50 per cent linoleic acid) is fed to an animal for long periods, then the composition of the adipose tissue triglycerides changes and approximates very closely to that of corn oil. Such changes as a result of dietary alterations may however take several months or even years to develop. This slow turn-over seems to conflict with the modern idea of a biochemically active adipose tissue. It may, however, be explained by postulating the existence of "compartments" of adipose lipid, some of which are in rapid exchange with the plasma lipids and dietary fat with others (perhaps the majority) in normal conditions acting as storage depots with a relatively low rate of exchange.

A characteristic feature of adipose tissue, often clearly visible to the naked eye, is its division into lobules by means of connective tissue septa. This is found especially in those regions (e.g. in the palm of the hand and the sole of the foot) where the fat acts as a shock absorber. In addition to these prominent collagenous septa, there is a well-developed reticulin network around each individual fat cell.

Adipose tissue has a rich capillary blood supply, a feature correlated with the biochemical activity of the tissue and with its function in acting as a heat generator.

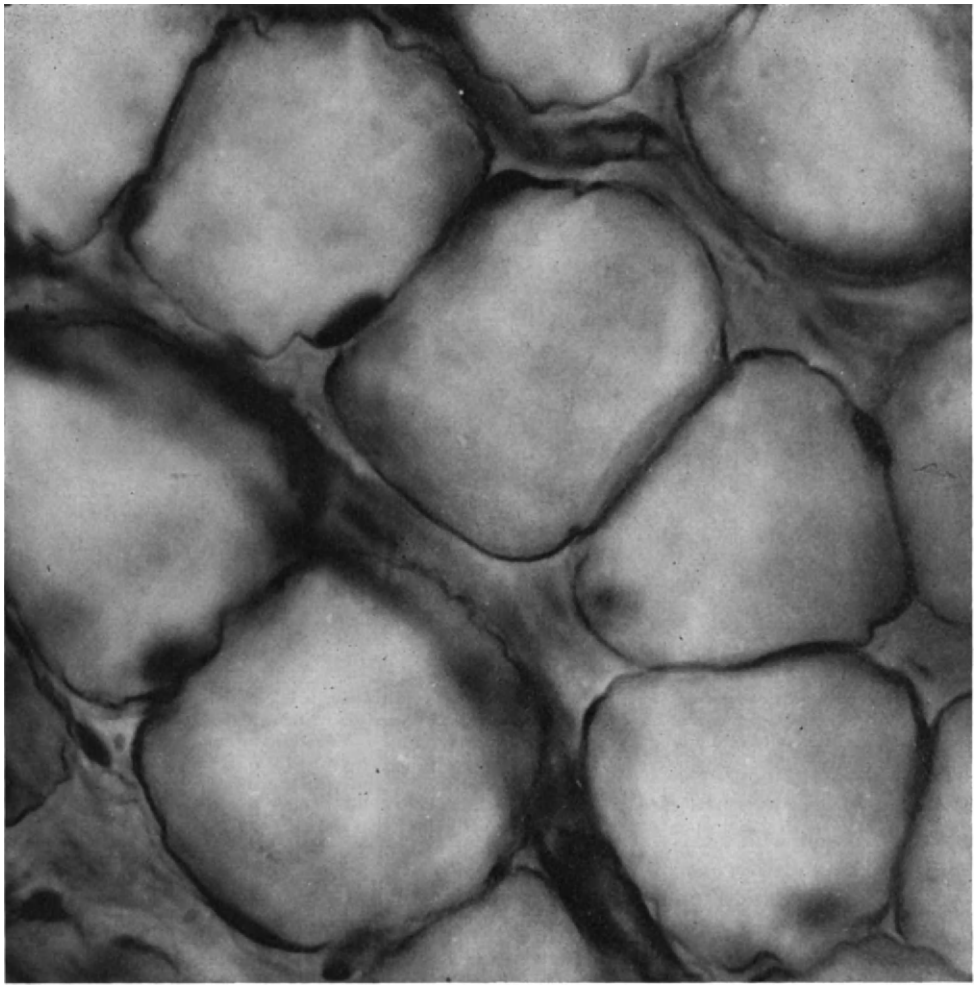

FIG. 7.1. The typical appearance of white adipose tissue cells when the material has been prepared by embedding in paraffin wax. The large triglyceride droplet has been dissolved away, leaving just the characteristic rim of cytoplasm and the peripheral nucleus. 20 μm.

With the E/M, the cytoplasm of white adipose cells is seen to contain only a small number of mitochondria, a small Golgi apparatus, some free ribosomes and a few profiles of smooth surfaced endoplasmic reticulum. The lipid droplet itself appears without structure and does not appear to be bounded by a unit membrane.

Chief Distribution

In the superficial fascia. There is a marked difference in the amount present in various regions of the body and there is also a marked difference in its distribution in the two sexes.

Omentum and mesenteries.
Perinephric region.
Yellow bone marrow.
As a packing in spaces such as the ischio-rectal fossa, the axilla and the bony orbit.

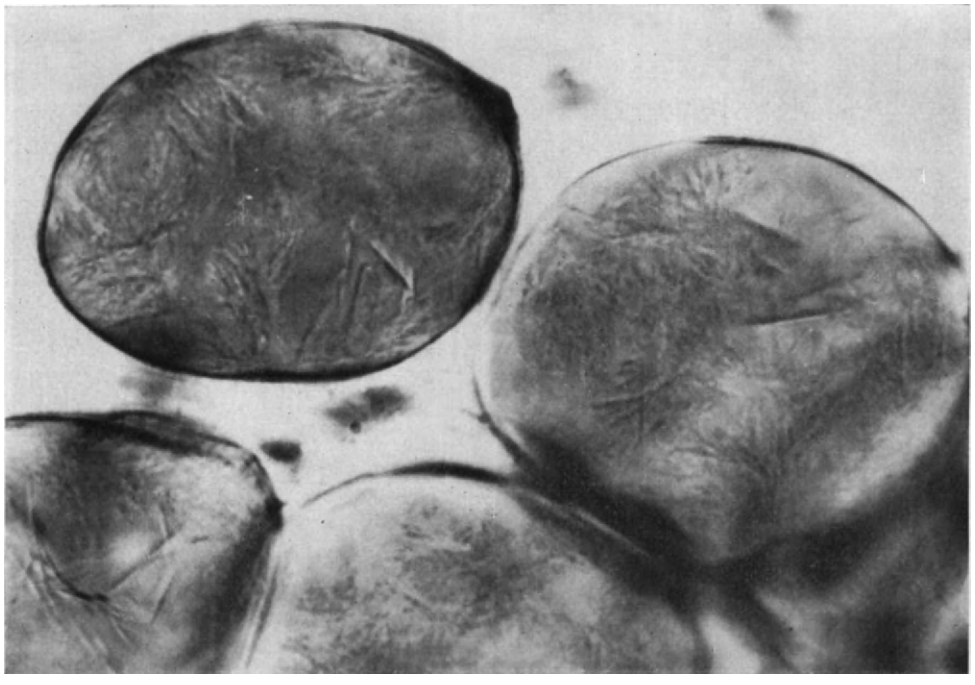

FIG. 7.2. White adipose tissue cells in the omentum prepared by a technique which preserves the triglyceride. This has been coloured by the use of a fat-soluble dye. The position of the nucleus can be clearly seen bulging the cytoplasmic rim outwards. 20 μm.

B. Brown (Multilocular) Adipose Tissue

Brown adipose tissue, as its name implies, is of a much darker colour than the typical white or yellow fat. It tends to occur in smaller cells in which characteristically there are many lipid droplets which never seem to coalesce into a single droplet (Fig. 7.3B). This existence of many small droplets rather than a single large one seems to be an adaptation for the provision of a large interface between lipid droplets and cytoplasm within the cell, so ensuring ideal conditions for the rapid metabolism of the fat. In consequence of this, although the nucleus may assume an excentric position, it never gets pushed to the periphery of the cell as is the case with white or yellow fat. When examined with the electron microscope brown adipose tissue cells are distinctive not only by virtue of their large numbers of fat droplets, but also because of the large numbers of mitochondria which seem to occupy most of the cytoplasmic volume. These mitochondria possess very large cristae which often extend across the whole thickness of the mitochondrial matrix. The mitochondria themselves are often seen to be pressed into close apposition to the surfaces of the fat droplets (Fig. 7.3C). There is little endoplasmic reticulum and only a few free ribosomes. Biochemical studies have shown that the mechanisms for oxidative phosphorylation cannot be demonstrated in the mitochondria of this tissue and examination with the E/M has shown that the elementary particles (thought to carry the electron transport sequence of enzymes) are absent from their cristae. This means that the energy released from esterification of the triglyceride cannot be used to form ATP but is instead immediately available in the form of heat.

As one might expect, the brown adipose tissue has an exceedingly rich blood supply, much more profuse than that of the white adipose tissue. This again is correlated with the function of the tissue as a heat generator, serving to distribute the heat energy to the underlying body tissues as a whole.

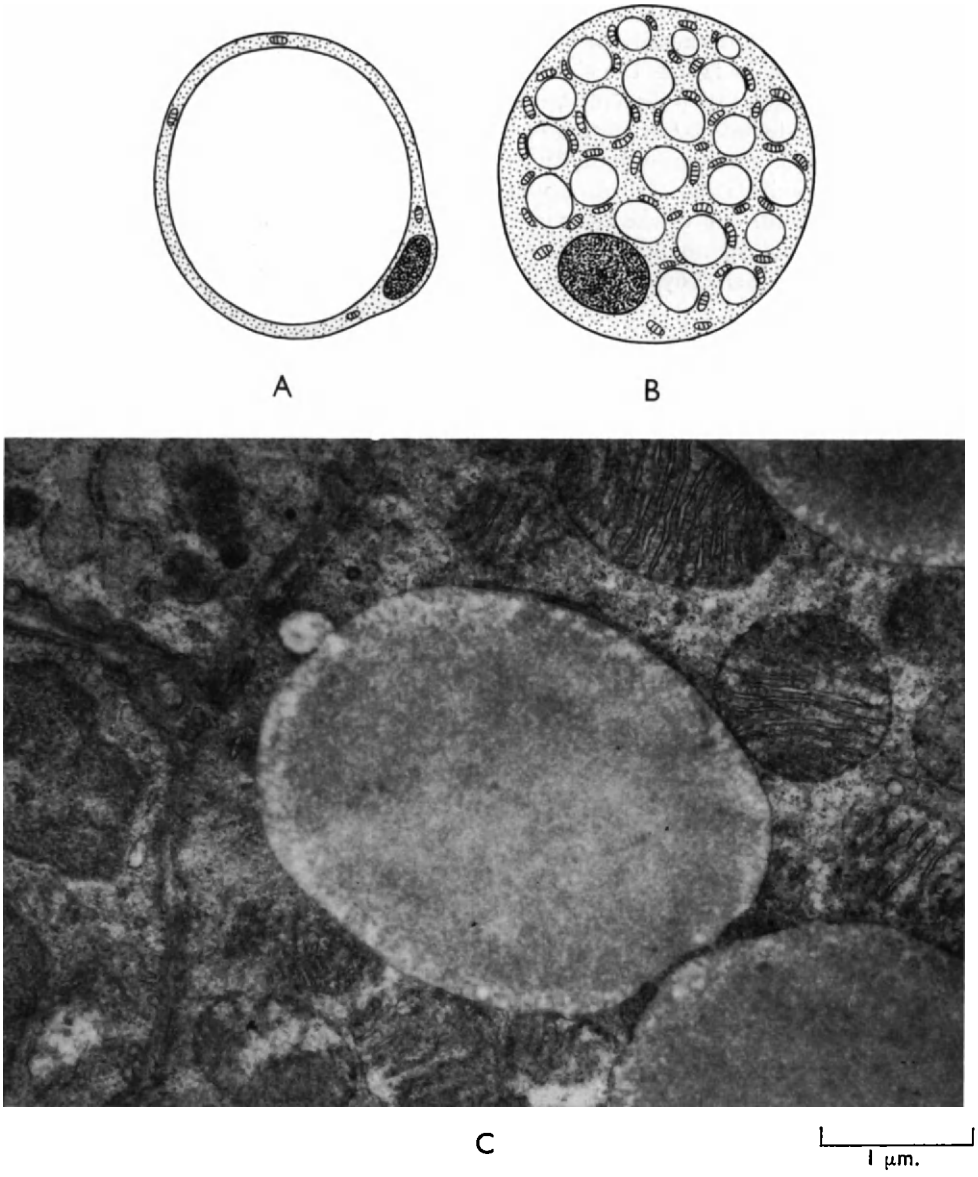

FIG. 7.3A. A drawing of the morphology of a white adipose cell. There is a single large fat droplet, few cytoplasmic organelles and a peripheral nucleus.

B. A drawing of a brown adipose tissue cell. There are many small, spherical droplets of triglyceride and many mitochondria in close apposition to them.

C. An electron micrograph of part of the cytoplasm of a brown adipose tissue cell. Parts of three lipid droplets are visible. Note also several mitochondria with very large and prominent cristae in close proximity to them.

Chief Distribution

Interscapular regions. Perinephric regions.
Axillary region (few nodes only).
Along the thoracic aorta.
In the mediastinum.

Recent work by Heaton has shown that this type of fat disappears gradually up to the age of 30 and more abruptly thereafter although the deeper regions (e.g. para-aortic and mediastinal) tend to retain it as late as the eighth decade of life.

In the adult brown fat is difficult to demonstrate histologically, because in the human adult rapid heat production is not required by this means and the droplets then tend to coalesce into a single droplet in the cell, so simulating the condition in white fat. In starvation masses of tissue containing multilocular fat cells appear in the sites where brown fat has been shown to exist in rodents. Again one of the two types of lipoma or fatty tumour resembles brown fat in its histology. These arguments tend to support the idea that both types of fat are present in the human even though they cannot be distinguished morphologically.

C. Development of Adipose Tissue

For many years adipose tissue has been regarded as a variant of connective tissue, in which the primitive mesenchyme cells or even the fibroblasts have developed fat droplets in their

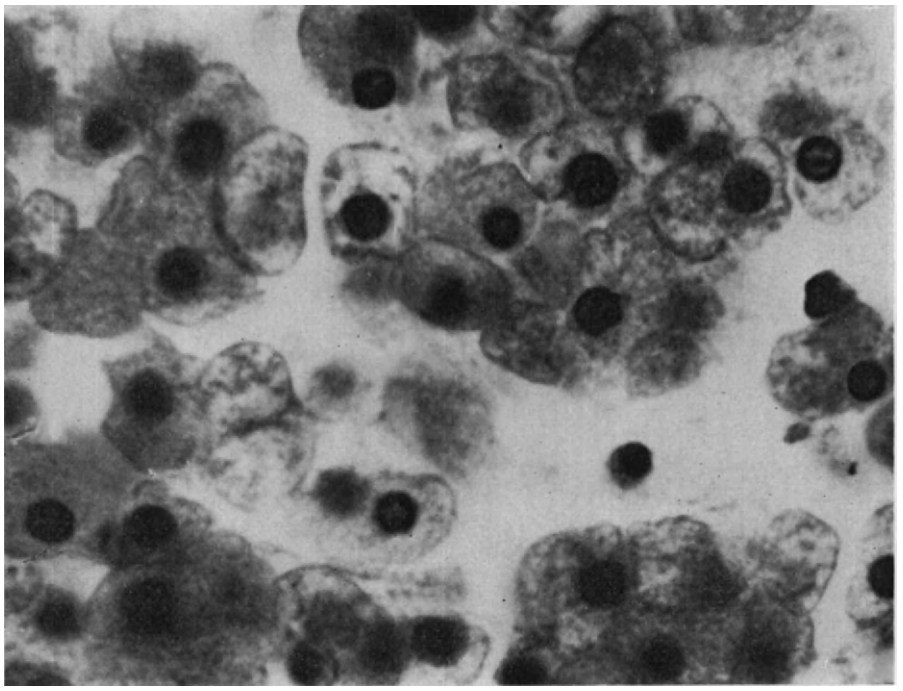

FIG. 7.4. Developing cells in a "fat island" of a 150 mm. human embryo. The lipid at this stage occurs as numerous small droplets in each cell (they have been dissolved in the preparation of this slide) and the nucleus has not yet assumed the typical peripheral position. 20 μm.

cytoplasm. This view, which stems from the work of Flemming, still has some support. A variant idea is that of Toldt, who holds that adipose tissue is derived from cells known as *lipoblasts*, which in turn arise from the common ancestral mesenchyme cell and have become concentrated in a special region of the embryo. Toldt believed that in starvation the depleted fat cells could revert to the lipoblast condition but not to the fibroblast state, whereas this was so with the previous theory. It has been suggested that both theories may hold good for the human embryo: accumulation of lipid in a fibroblast cell producing white adipose tissue whilst the accumulation of lipid in lipoblasts was responsible for the development of the brown

adipose tissue. If fat continues to accumulate in the lipoblasts then ultimately droplet fusion does occur and the brown fat then loses its distinguishing histological feature.

Wasserman has devoted much study to the embryogenesis of adipose tissue and he believes that "fat islands" are clearly differentiated in the embryo, where they are produced from the proliferation of mesenchymal cells forming the adventitia of blood vessel walls. The appearance of a fat island in the subdermal tissues of an embryo is illustrated in Fig. 7.4. Wasserman is convinced that no conversion of connective tissue cells into the cells of adipose tissue ever occurs.

D. Functions of Adipose Tissue

(1) **Mechanical Packing and Shock Absorption.** The widespread occurrence of adipose tissue exemplifies its value as a means of mechanical packing around various organs of the body. Good examples of this are seen in the perinephric fat around the kidney which plays a large part in the maintenance of this organ in its position, and in the bony orbit where the eyeball is stabilized in its fascial sling by the presence of the fat. Almost any potential space in the body where distension of some neighbouring organ might be required has its quota of white adipose tissue. The layer of subcutaneous adipose tissue also plays a part in the smoothing out of the body contours and in the furnishing of sexual dimorphic contours. In some places the subcutaneous adipose tissue acts primarily as a shock absorber, e.g. in the buttocks, the sole of the foot and in the palm of the hand. In these situations the fat tends to be aggregated into much more prominent lobules with more fibrous connective tissue in between them than in other areas. At the same time the degree of vascularity tends to be less in these areas and it is reported that even in cases of extreme emaciation where most of the subcutaneous lipid has been mobilized, the lipid in these shock absorbing pads is still intact.

(2) **Energy Storage.** Adipose tissue is one of the chief storage sites for the body as triglyceride is a very economical way of storing energy. The triglyceride of the adipose tissue may be synthesized either from the chylomicrons of lipid or fatty acid absorbed from the gut or synthesized *de novo* from glucose in the liver or in the adipose cells themselves.

If the glucose supply is continuous, then little triglyceride is broken down again; if, however, as is more common, there is an alternation of fasting and feeding, then the breakdown of adipose triglyceride may be greatly accelerated during the periods of fasting. This helps to preserve a balanced supply of energy to the body.

The process of lipolysis is under the direct control of hormones from the adrenal cortex, the thyroid, and of insulin from the pancreatic islets. If the level of the latter hormone falls, as in diabetics, carbohydrates cannot easily be utilized as prime energy sources and fat is then substituted for them.

As there is a rich autonomic innervation of adipose tissue it might be expected that the mobilization of lipid from the reserves in this tissue would be under nervous control. This has been shown to be the case.

(3) **Thermal Control.** It has been suggested in many publications that the localization of large amounts of adipose tissue in a thick layer underneath the skin was a reflection of the importance that this tissue was playing as an insulating blanket, preventing the loss of body heat. The functioning of adipose tissue in this manner would presuppose a fairly low coefficient of thermal conductivity; in fact measurements in recent years have shown that the thermal conductivity of the lipid in adipose tissue is approximately the same as that of olive oil. This is quite high and not what would be expected if the fat were acting as a prime insulating layer. If the layer is thick, as it often is, then there will undoubtedly be some insulating action but it might be questioned whether this is in fact its prime role. Cahill in a recent publication states that the insulating capacity of the fat layer is equal to that of a layer of leather of equal thickness, which is about ten times less than that of an equal thickness of feathers or of hair.

Metabolic and biochemical studies suggest that the adipose tissue is in fact more impor-

tant as a heat generator, the turnover and esterification of triglyceride resulting in the liberation of heat which is passed to the underlying body tissues by means of the rich vascular supply. This is certainly true of brown adipose tissue. This latter is very abundant in those species which hibernate. When they are awakening from dormancy it has been shown that the brown adipose tissue is rapidly metabolized (under the influence of noradrenaline) to provide the heat which warms the body up to the point at which the muscular contractions of shivering can take over the process. At the same time as heat is produced, large quantities of oxidizable substrates are liberated into the circulation and made available for use by other organs and tissues.

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CHAPTER 8

CARTILAGE

Cartilage is a translucent, firm tissue of considerable rigidity, well adapted for weight bearing; it is closely associated with the bony skeleton, most of which was originally represented by cartilage in the foetus. Like other connective tissues, cartilage consists of cells and intercellular matrix that contains fibres: the intercellular matrix is responsible for those properties of cartilage that enable it to withstand a considerable degree of pressure, tension and torsion. At the same time it shows a considerable degree of elasticity. In the costal cartilages, for example, this helps to restore the resting shape of the thorax and so assists in the expiratory phase of respiration. Fully developed cartilage is supplied by no demonstrable blood vessels, lymphatics or nerves.

The character of the intercellular matrix varies, and three types of cartilage can be distinguished: hyaline (or glass-like) cartilage, fibrocartilage and elastic cartilage. Articular cartilage, which covers the bone surfaces in synovial joints, represents a slightly modified type of hyaline cartilage in which the fibres in the intercellular matrix are arranged in a regular rather than a random pattern.

A. Hyaline Cartilage (Fig. 8.1)

In the foetus most of the skeleton is laid down as hyaline cartilage: subsequently this is largely replaced by bone (see Development of bone), but hyaline cartilage persists throughout life in the costal cartilages at the ventral ends of the ribs, and in the cartilages of the nose, larynx, trachea and bronchi: in the latter situations it maintains a patent lumen in the respiratory passages by preventing obliteration through pressure.

Hyaline cartilage is closely covered with a tough and vascular fibrous membrane, the *perichondrium*; this is absent over the articular cartilage in joint cavities. The cartilage consists of cells and of a solid intercellular matrix; it is very flexible, somewhat elastic and semi-transparent. It is supplied by no blood vessels or lymphatics of its own, hence all nutrition must be by diffusion through the matrix. Large masses are, however, traversed by blood vessels running in cartilage canals en route to other destinations. In the early stages of cartilage development the cartilage canals are probably of nutritive significance but recent studies by workers such as Lutfi and Wilsman and van Sickle suggest that the primary role of the cartilage canals is to provide a source of stem cells for further interstitial cartilage growth. These stem cells are of mesenchymal origin and are found in the canals together with blood vessels. No nerve fibres have been demonstrated supplying the substance of cartilage.

The Cells (chondrocytes). The cartilage cells are large, often over 40 μm. in diameter and usually spherical. They lie in capsules and completely fill the spaces or lacunae in the matrix in which they lie: in the adult the cells are not branched, although in the embryo they commonly possess processes. There is a large nucleus with one or more nucleoli: the cytoplasm contains glycogen granules, fat droplets and sometimes pigment, while vacuoles are frequently present. The cells are very sensitive to most reagents and readily undergo distortion during fixation due to the solution and removal of their contents. In the mature state the chondrocytes do not divide. With the E/M large amounts of rough-surfaced endoplasmic reticulum may be seen in the cytoplasm. The Golgi apparatus is often very large with well-developed vacuoles; numerous particles of glycogen may be seen as well as mitochondria.

On the border between cartilage and perichondrium are found ordinary undifferentiated fibroblasts. The arrangement of the cells in the cartilage is characteristic. In the peripheral layers beneath the perichondrium or near a free joint surface the cells are flattened in a plane

parallel with the surface (Fig. 8.2). Often the cells of a mass of cartilage are arranged in groups, which often consist of two, four or eight cells; this is due to the division of a single parent cell into two daughter cells which then divide again, and so on: the daughter cells tend to remain near together because of the nature of the matrix, and thus the cells become ovoid and flattened on adjacent sides forming so-called "cell-nests" (Fig. 8.3).

The Intercellular Matrix. The ground substance appears homogeneous when fresh and has a high water content (*c.* 70 per cent); it stains with basic dyes due to the presence of chondromucoprotein, a polymer of a mucoprotein together with chondroitin-4-sulphate and chondroitin-6-sulphate. Small amounts of keratan sulphate may also be present. These sulphated

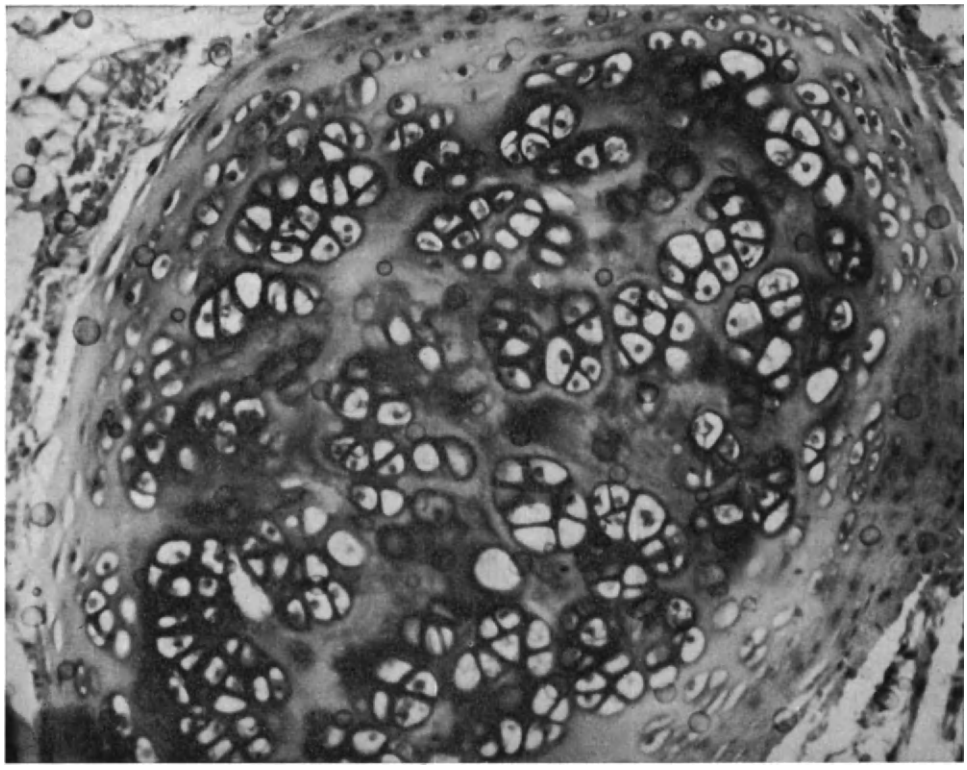


FIG. 8.1. The typical appearance of hyaline cartilage from one of the costal cartilages. Notice the "cell-nests" of mature chondrocytes surrounded by the darkly staining capsules. The more flattened cells below the perichondrium are less mature.

mucosubstances are responsible for the metachromatic staining of the cartilage matrix with some thiazine dyes and for the strong uptake of such dyes as Alcian Blue 8 GX. The matrix gives particularly intense staining reactions around the walls of the cell lacunae: these "envelopes" for the cells are the cell capsules (see Figs. 8.1 and 8.3) and represent the most recently formed intercellular matrix. The subperichondrial layers stain strongly with acid dyes.

When boiled in water cartilage slowly dissolves, giving rise to cartilage glue or chondrin, which contains gelatin, chondromucoprotein and other albuminous substances. The intercellular matrix is permeated with a dense felt work of very fine collagen fibres, the apparent homogeneity when fresh being due to the fact that the matrix covering and binding the fibres has the same refractive index as that of the fibres. Polarization microscopy and the E/M show that the fibres have a preferential orientation which is coincident with the directions of

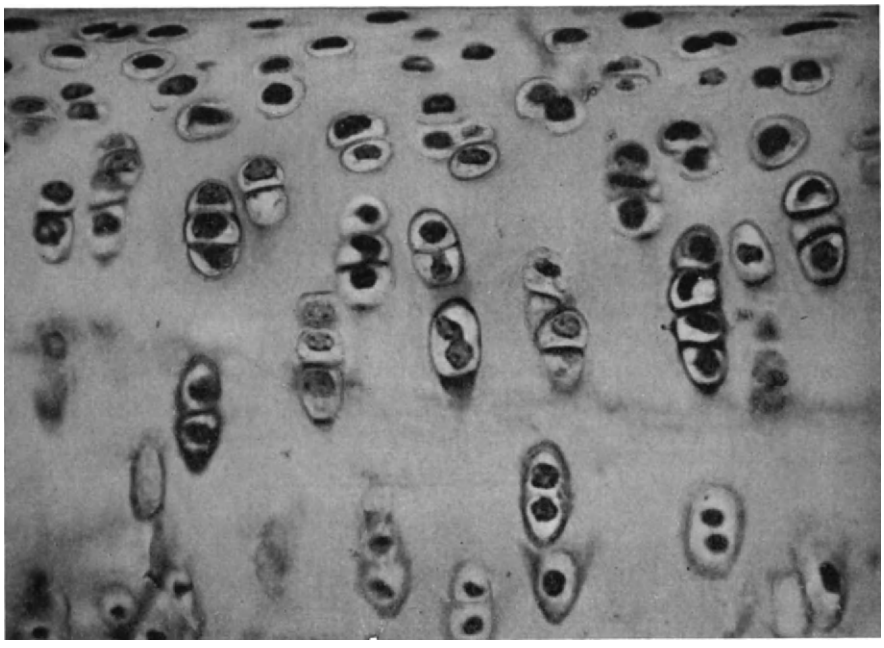


FIG. 8.2. Vertical section through articular cartilage. The free surface is at the top of the picture. Note how the cells are flattened in a plane parallel to the surface.

40 μm.

maximal stress. This phenomenon is especially marked in articular cartilage. Much of the matrix can, however, be removed by maceration or by tryptic digestion and the fibres are then easily seen. The central parts of the cartilage mass are subject to pressure and here the chondromucoprotein is particularly concentrated, while the collagen fibres are more numerous in the peripheral layers where the applied force is one of pull.

As there are no canaliculi in the ground substance the cells are nourished by fluid exchange with the intercellular matrix, the nutritive fluid being derived from the blood vessels of the perichondrium. With articular cartilage diffusion of nutrients from the synovial fluid is thought

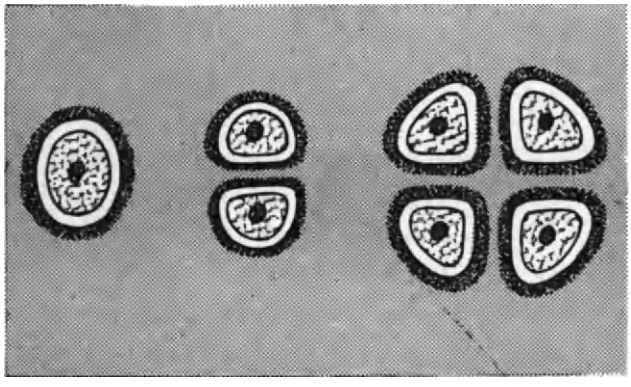


FIG. 8.3. A diagram to show the development of a "cell-nest" of cartilage cells by two mitotic divisions of the single cell (on the left) encased in matrix. After division the four daughter cells (right) assume their characteristic shape due to compression by the matrix. The capsule (the most recently formed matrix) around the cells is shown in dark stipple.

to be of importance. In the larger cartilages, however, the system of canals containing blood vessels en route to other sites must assist in the supply of nutrients, especially in the early stages of development.

Chief Distribution of Hyaline Cartilage

Costal cartilages.
Cartilage of nasal septum.
Some laryngeal cartilages (e.g. thyroid cartilage).
Tracheal rings.

Articular cartilage of synovial joints and epiphyseal cartilage plates of growing long bones represent modified forms of hyaline cartilage.

B. Fibro-cartilage (Fig. 8.4)

This type of cartilage is found wherever great strength, combined with flexibility and rigidity, is required. It is well adapted to withstand shearing forces.

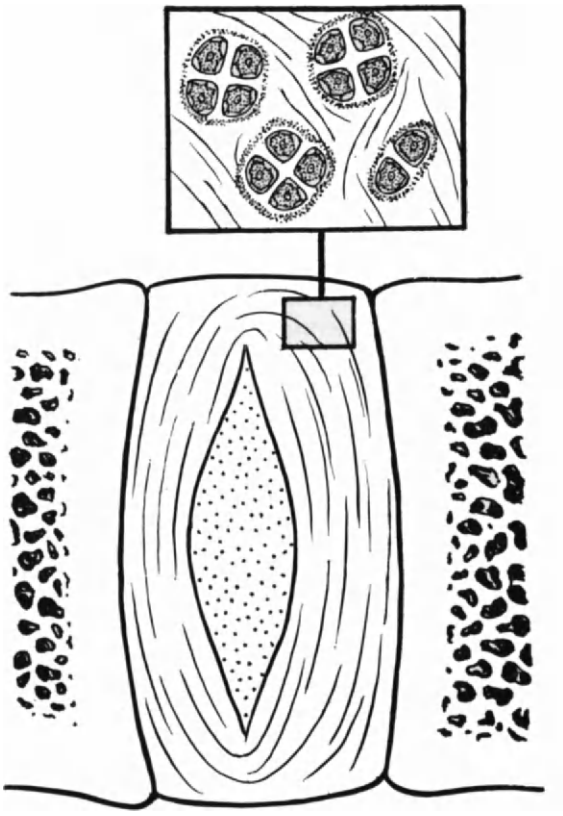


FIG. 8.4. Fibrocartilage occurs in the intervertebral disc between two adjacent vertebral bodies. Cartilage "cell-nests" are present, but the matrix between them contains large bundles of collagen fibres which are clearly visible (inset). The nucleus pulposus of the disc is shown in stipple.

Microscopically, white fibro-cartilage looks very like dense fibrous tissue, but the cells are typical cartilage cells in capsules, and not stellate tendon cells. The intercellular matrix contains thick bundles of collagen fibres running parallel with one another, and separated by narrow bands of non-fibrous matrix in which lie the rounded cartilage cells, often in pairs.

There is no perichondrium and the fibrocartilage merges into the surrounding dense connective tissue of the capsules and ligaments of joints: it really represents a transition between connective tissue and hyaline cartilage.

Chief Distribution of Fibrocartilage

Intervertebral discs.
Pubic symphysis.
Linings of many tendon grooves in bones.
In the attachments of some tendons.
Interarticular ligaments and joint discs.

C. Elastic Cartilage (Fig. 8.5)

Elastic cartilage differs from hyaline cartilage in that the ground substance is pervaded by a network of branching and anastomosing elastic fibres. The typical cartilage cells lie within

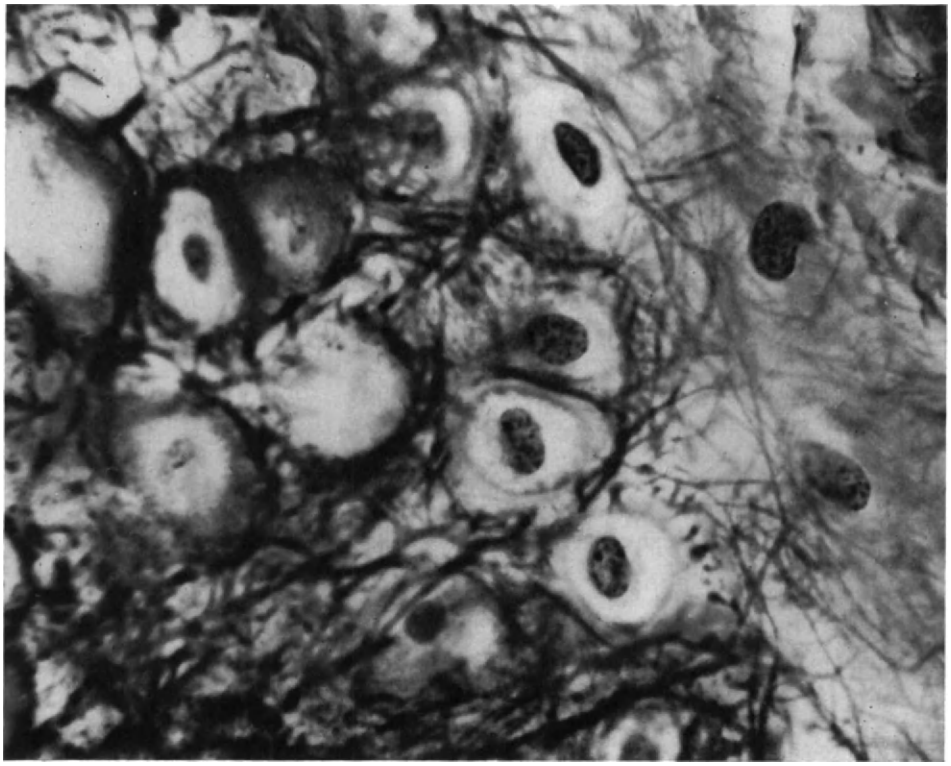


FIG. 8.5. Elastic cartilage in the pinna of a rabbit's ear. The preparation has been stained to show the branched elastic fibres in the cartilage matrix. These are especially dense around the cell capsules. 20 μm.

capsules and the elastic network is often especially dense immediately round the cells. The elastic fibres at the periphery are directly continuous with those of the perichondrium. The intercellular matrix contains, in addition to the elastic fibres, some fine collagen fibres.

This type of cartilage is found where flexibility is required, together with ability to recover shape after deformation.

Chief Distribution of Elastic Cartilage

External ear.
External auditory meatus.
Pharyngo-tympanic tube.
Epiglottis.
Some laryngeal cartilages (corniculate and cuneiform).

Regeneration of Cartilage

Hyaline cartilage does not undergo independent repair because the mature cartilage cells are incapable of division. Any defect is made good by invasion from the perichondrium or nearest fascia and by deposition of connective tissue. Some of the fibroblasts of this tissue then round up and are transformed into cartilage cells, while the intercellular matrix becomes changed into the typical ground substance of the cartilage. Thus it is not surprising that in repaired cartilage it is quite common to find elastic and white fibres in the matrix. Sometimes the repair stops short with the filling of the gap by dense fibrous tissue.

Regressive Changes in Cartilage

In old age regressive changes are frequently found, particularly in thick masses of cartilage: this is probably due to the poor nutrition of the cells. A decrease in the quantity of the intercellular matrix is apparent, together with a decrease in its basiphilia and in its high water content. Calcification commonly occurs in scattered areas of hyaline cartilage only, the calcium salt being deposited at first as crystals in the neighbourhood of the cells.

Development of Cartilage (Fig. 8.6)

Cartilage develops, like other connective tissues, from the embryonic mesenchyme. An aggregation of mesenchymal cells occurs, together with the withdrawal of their cytoplasmic processes. Intercellular matrix is secreted by the cells which then quickly acquire the characteristic appearance of cartilage cells, and become separated from one another by increased formation of the intercellular matrix. Collagen fibres are deposited in the matrix, but they soon become invisible as they are covered up by the homogeneous mass. The cell capsules become differentiated along the surface of contact between cells and matrix: after this the intercellular substance continues to increase in amount, so that the cartilage comes to consist of cells scattered in a homogeneous matrix and surrounded by perichondrium of connective tissue. E/M autoradiographic studies, both with S^{35} and with tritiated proline, have shown conclusively that the collagen fibres and the chondromucoprotein of the matrix are both secreted by the chondrocytes.

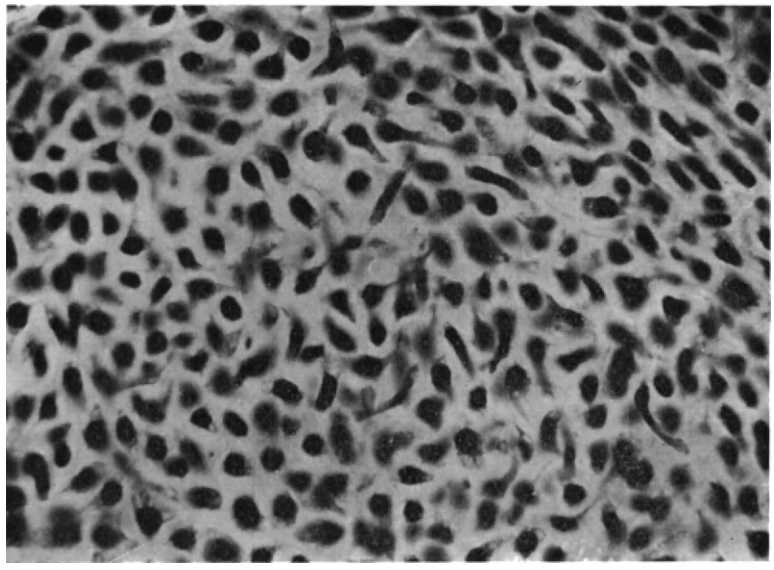
Continued growth occurs in two ways:

(*a*) **Interstitial Growth.** This occurs mostly in young cartilage. The cartilage cells divide into two and each of these may divide again so forming clusters of 2, 4 or 8 cells: the cells form new intercellular matrix and so become separated from one another, each surrounded by its own capsule. In a patch of cartilage the youngest cells are in the middle, the older cells being pushed outwards so that the senescent cells are more crowded.

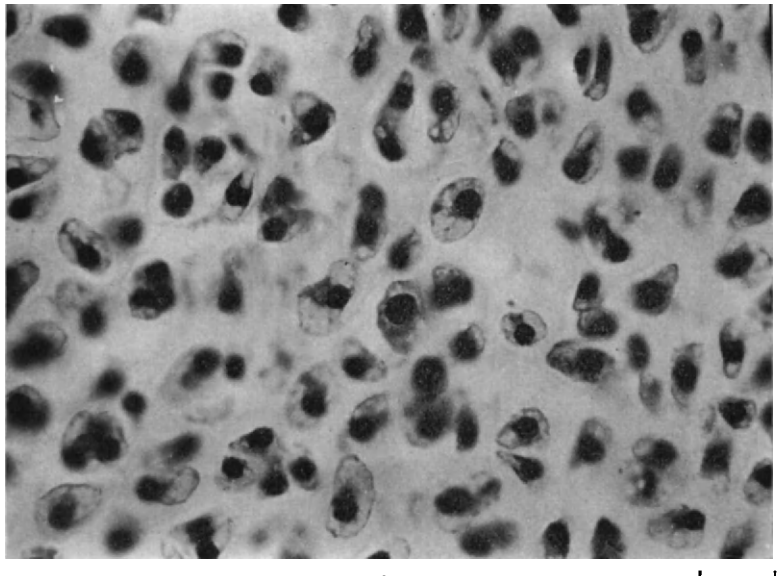
(*b*) **Appositional Growth.** The increase of the cartilage due to interstitial growth causes a mechanical stretching of the surrounding perichondrium. The innermost fibroblast cells of this perichondrial connective tissue become transformed into cartilage cells, the intercellular matrix acquires the appearance of that of typical cartilage, and so the cartilage increases by accretion from the periphery.

A large amount of the cartilage formed in the embryo is later replaced by bone.

Fibro-cartilage is developed in the same way as hyaline cartilage. During development



A



B

40 μm.

FIG. 8.6A. An early stage in the development of hyaline cartilage. Mesenchyme cells have aggregated and just begun to round off; matrix secretion has not yet begun.

FIG. 8.6B. A later stage in the development of hyaline cartilage. The cartilage cells are now separated from one another by the newly secreted matrix.

fibres, either collagen or elastic, are formed in the intercellular matrix and gradually increase in amount. In some parts white fibro-cartilage is formed by transformation of the dense white fibrous tissue of tendon or ligament, the cartilage cells being formed directly from tendon cells, which are themselves of fibroblastic origin.

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CHAPTER 9

BONE

Bone, like other types of connective tissue, consists of cells, fibres and an interstitial matrix, but it is very highly specialized in that it is rendered rigid by the impregnation of its ground substance with calcium salts. The skeleton contains about 97 per cent of the total calcium of the body. About 65 per cent of the weight of fresh bone is due to its inorganic constituents, calcium phosphate composing 85 per cent of this and calcium carbonate 10 per cent, while calcium fluoride and magnesium chloride are also present. The salts can be dissolved out by treatment with mineral acids or removed by treatment with a chelating agent such as ethylene diamine tetra-acetic acid, and the remaining decalcified bone can then be examined by the usual histological methods.

Bone is a substance which has a very high mechanical strength combined with a remarkably low weight for its strength. It is organized at both macroscopic, microscopic and ultramicroscopic levels so that the greatest possible mechanical strength is obtained with the minimum use of material. Although bone is often regarded in terms of the inert prepared skeletal material usually seen in anatomical teaching laboratories, it should be remembered that bone is a relatively plastic component of the body and one which has a marked metabolic activity, especially with respect to calcium ions.

The bony skeleton serves as an internal supporting system for the body, it acts as a source of origin and insertion for the muscles, especially those serving locomotion, and it acts to give mechanical protection to vital viscera such as the brain, heart and lungs and plays a vital role in the mineral metabolism of the body.

Bone differs from other hard skeletal material in the body (calcified cartilage) in that (i) it is very vascular (ii) it may possess an extensive canalicular system throughout its matrix (iii) it can increase only by appositional growth because it is rigid, and (iv) it is permanent but shows some plasticity and local reconstructive ability.

Bone is also a body depot for calcium salts necessary for the maintenance of the equilibrium of the internal medium: the calcium is continually withdrawn from this depot and the loss is made good by an equivalent fixation of calcium, as the new formation of bone substance replaces that lost by physiological resorption. This process is under endocrine control (see p. 229).

Gross Structure of Bone

Two types can be distinguished, *spongy* (*or cancellous*) bone, and *compact* (*or dense*) bone like ivory (Fig. 9.1); the former has large vascular channels whereas in compact bone the vascular channels are small, often of microscopic size. If a long bone is cut longitudinally the epiphysis is seen to consist of a spongy arrangement of fine anastomosing bony spicules, with marrow contained in the meshes and a thin outer covering of dense white bone. On approaching the diaphysis or shaft the small spaces of the spongy bone become continuous with the large central cavity that contains marrow, and the compact bone forms a thick outer wall. The architecture of the spongy bone directly reflects the degree of mechanical stress to which it is subject. Along the lines where such stresses are greatest, thickenings or *trabeculae* of the bony spicules are found. These may be clearly seen by the naked eye in a section of, say, the femoral head or the calcaneum and they appear as areas of extra density on an X-ray film. The trabeculae may be divided into some related to tensile strains and others due to compression forces. Although some authorities have discounted the effects of mechanical forces upon the development of the trabeculae the general consensus of opinion seems to accept this hypothesis. Two striking pieces of evidence which may be quoted in its support are that the trabeculae do not appear in

the lower limb of the human infant until it begins to walk and that if an ankylosis of a joint takes place or if there is a badly set fracture, then the pattern of the trabeculae may change completely to conform with the altered mechanics of the skeleton. In the shaft of the long bones the wall is chiefly of compact bone, but the inner layer immediately lining the central cavity is often spongy. The flat bones of the skull consist of two plates of compact bone with a layer of spongy bone between them (diploë). The short bones are usually spongy, with a thin covering of compact bone.

Every bone, except at the articular surfaces, is covered by a vascular connective tissue layer, the *periosteum.*

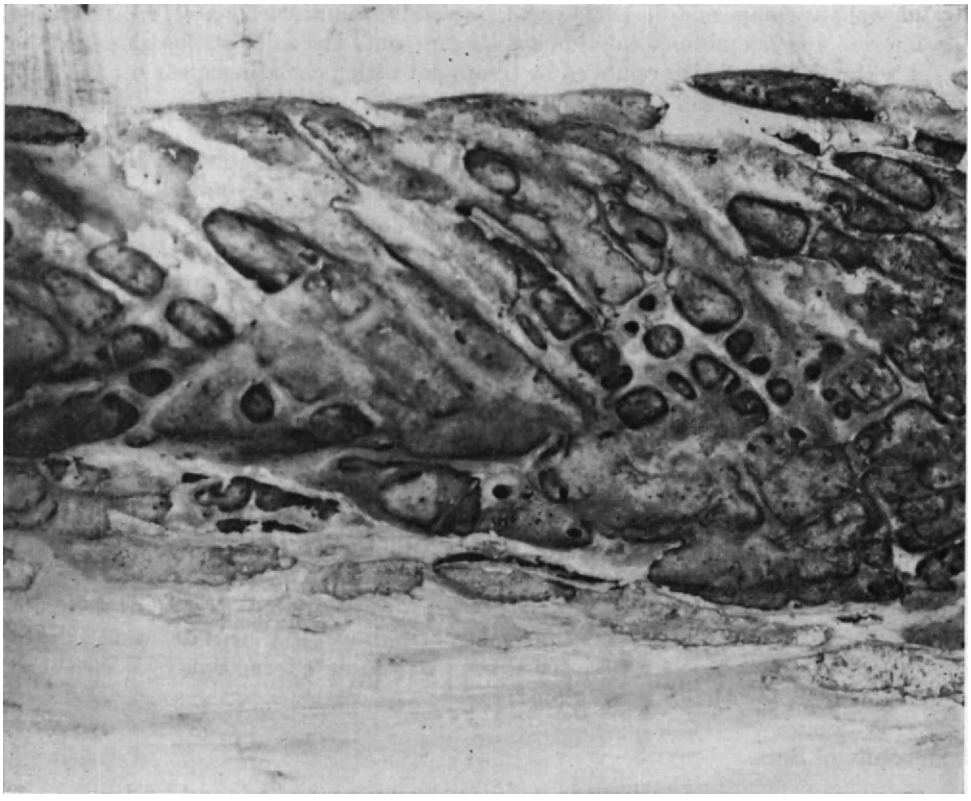


FIG. 9.1. A photograph of the internal aspect of the shaft of the human femur. Compact bone is at the top and bottom of the picture, whilst the endosteal surface of the marrow cavity is spongy bone; the trabeculae are clearly visible.

Microscopic Structure

Two main types of bone may be recognized at the microscopic level; these are *woven* (coarse-fibred) and *lamellar* (fine-fibred) bone, either of which may be compact or cancellous when studied with the naked eye. The diaphysis or shaft of developing long bones in the late foetus, for example, is woven compact bone, whilst the developing mandible (and also the callus formed during the repair of adult fractured bones) belongs to the woven cancellous variety.

The cortex of an adult bone is typically lamellar compact bone, whilst the bone nearest the medullary cavity is of the lamellar cancellous type (Fig. 9.1).

Woven bone is composed of irregularly arranged plates of spicules of bone forming a three-dimensional network with large vascular spaces in between. Within the bony spicules the

collagen fibres are arranged in coarse bundles which ramify and interweave in all directions. The cells of the bone (or *osteocytes*) are scattered irregularly amongst the matrix. Woven bone is nearly always deposited first during osteogenesis but it is short-lived and sooner or later it is resorbed and replaced by lamellar bone. The lamellar type of bone is more highly organized, being composed of *lamellae* or layers, which consist of calcified matrix containing the highly-orientated collagenous fibres. It should be noted, however, that the calcified matrix is continuous, the lamellation being in a sense an optical artifact due to an alteration in the orientation of the collagen fibre bundles in successive lamelae. Between and amongst the lamellae are the *bone cells* (*osteocytes*), flattened connective tissue cells which occur singly, and lie in spaces called *lacunae*: a bone cell fills its lacuna, which is usually ovoid in shape. Many fine projections arise from the bone cells (Fig. 9.2) and these penetrate into corresponding projections of the lacunae. From these latter arise innumerable fine passages, the *canaliculi*, which penetrate the

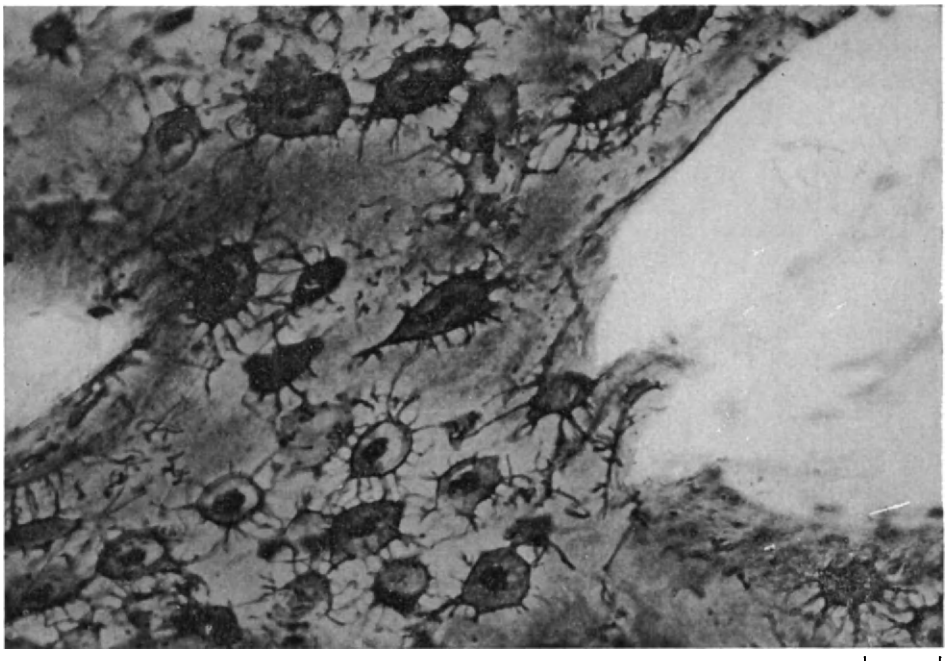


FIG. 9.2. Osteocytes in developing bone of an 18 week foetus.

hard interstitial matrix, branching and anastomosing, and connecting all the lacunae into one continuous system.

The *interstitial matrix* of the lamellae appears homogeneous under the optical microscope, but in reality it contains fine collagen fibres like those of the cartilage matrix as well as the crystals of bone salt. The interstitial matrix that forms the walls of the lacunae and canaliculi is specialized in that it is extremely resistant to destructive agents; it corresponds to the capsule of cartilage cells. The characteristic hardness of bone is due to the inorganic components in the interstitial matrix whilst the organic materials provide the associated resilience and toughness. If the organic matrix is removed (for example by calcination) then the inorganic remnant of the bone is found to be very brittle; on the other hand, if the inorganic materials are removed then the appearance of the bone is little changed but it can be shown to be so flexible and soft that the bone can easily be distorted and cut with a knife. A long slender bone such as the human fibula may even be tied into a knot after it has been decalcified.

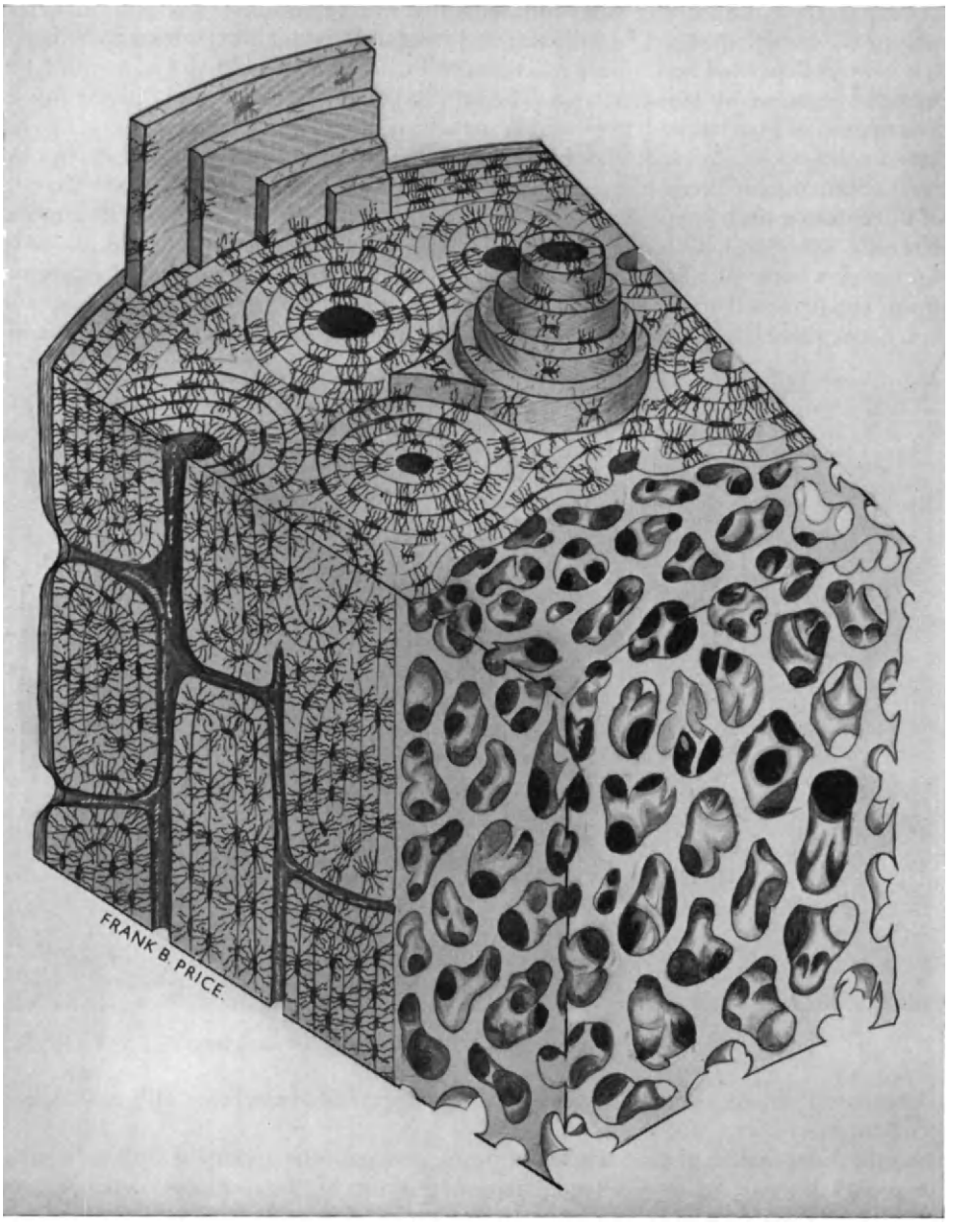


FIG. 9.3. A diagrammatic view of part of the shaft of a long bone. The lamellar compact bone of the shaft is surrounded at the periphery by circumferential lamellae in which the bundles of collagen fibres may be seen to run in different directions. Parts of six osteones may be seen, each with its central Haversian canal; cross connections between osteones are established by Volkmann's canals. A layer of spongy bone lines the marrow cavity.

The organic matrix consists largely of a protein polysaccharide complex with chondroitin-4-sulphate forming the largest component as in the case of cartilage. Some hyaluronic acid and sialic acid are also present, together with other polysaccharide constituents in small amounts. There are numerous bundles of collagenous fibres in the lamellae, embedded in the

interstitial matrix; these fibres are predominantly orientated in a spiral fashion, the direction and pitch of which is different in each successive lamina. This is the explanation for the peculiar birefringence which cross-sections of bone exhibit when they are examined under the polarizing microscope (Fig. 9.5).

The inorganic bone-salt is found in the form of needle-like crystals which are deposited in a regular fashion in close apposition to the collagen fibrils. X-ray diffraction studies have shown that the bone-salt is mainly hydroxy-apatite $Ca_{10}(PO_4)_6OH_2$ but carbonate and citrate ions are also present. If there is an appreciable concentration of fluoride ions in the drinking water, then it is possible for F^- to substitute for the OH^- in the apatite molecule. The apatite crystals are about 20–40 nm. in length and between 1·5–3 nm. in thickness. Magnesium and sodium

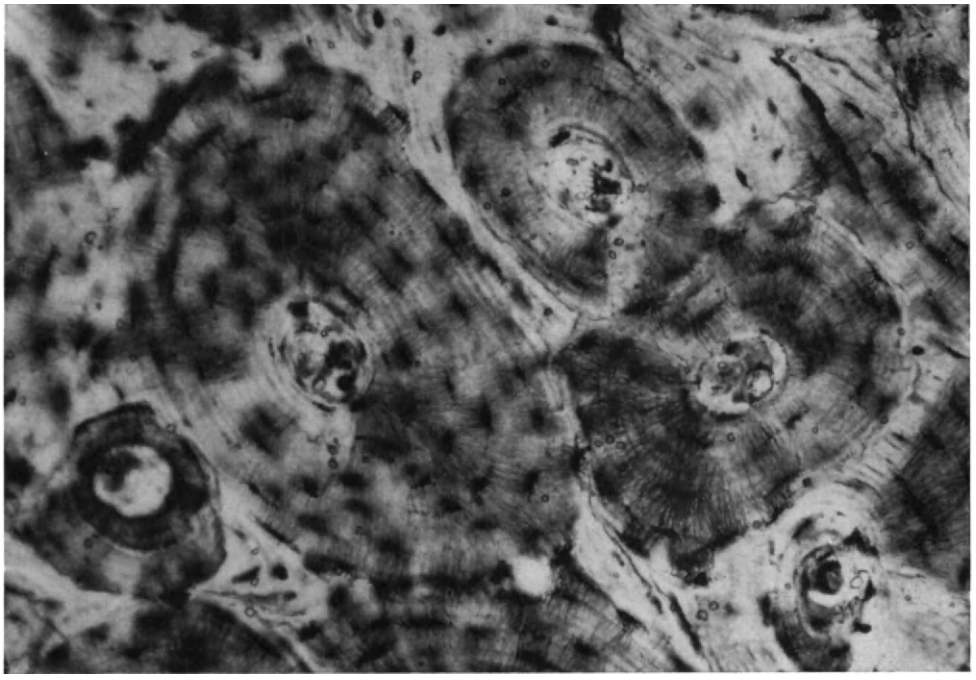
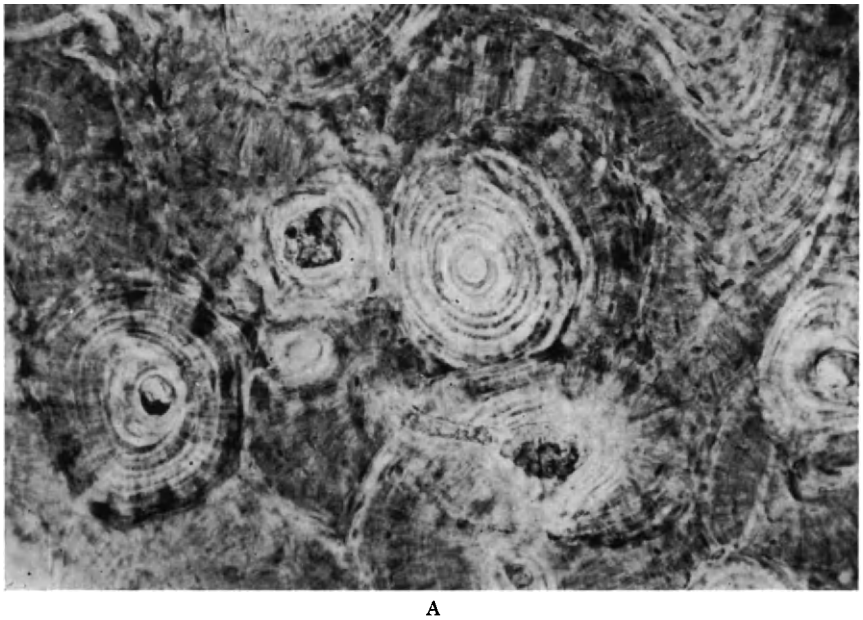


FIG. 9.4. A ground section of lamellar compact bone. The osteones or Haversian systems are seen in transverse section, with their large central canals, lacunae and canaliculi. These all appear dark in the photograph because they have become filled with debris produced during the grinding of the section. 60 μm.

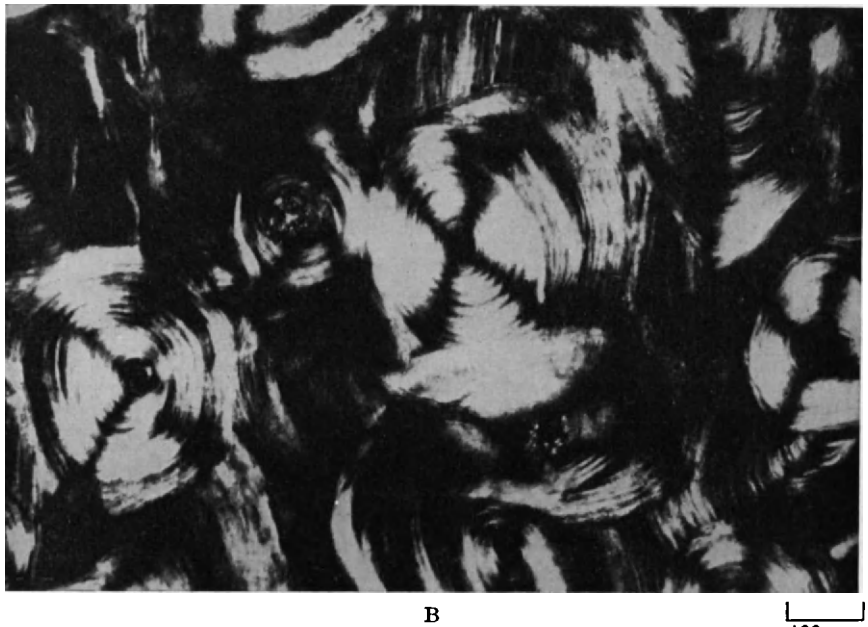
ions are always present in the bone mineral and in fact approximately 46 per cent of the body sodium is found in this site.

Certain radioactive isotopes, termed *bone-seeking isotopes*, may become incorporated into the bone. The radioactive isotope Ca^{45} may substitute for the normal isotope or radioactive Sr^{90} may be found in place of calcium in the bone salt. P^{32} may also be found incorporated into the mineral whilst other isotopes, for example Pu^{259}, may be bound by the matrix. These radioactive isotopes which are produced by the fission of uranium or as a result of atomic explosions will, if incorporated into the bone, act as a radioactive source irradiating the marrow and soft parts and may eventually give rise to leukaemia or to tumours of the bone. Such radioactive bone-seeking isotopes are of use in studies on bone metabolism and growth as their presence may be detected by means of autoradiography.

The arrangement of the bony tissue in lamellae is explained by the way in which bone is laid down by the addition of new layers on the surface of that already formed. If a section of



A



B

100 μm.

FIG. 9.5A. A thick ground section of bone similar to that in Fig. 9.4 photographed by direct microscopy.

FIG. 9.5B. The same field but photographed in polarized light. Notice the characteristic birefringence (which appears as bright areas) shown by three of the osteones. From this appearance the regular arrangement of the collagen fibres in the lamellae may be inferred.

compact bone is examined it is seen that the lamellae are arranged in concentric rings which surround tubes, the *Haversian canals*, which contain blood vessels and nerves. The canals run parallel to the long axis of the bone and are united to each other by oblique communications: some of the canaliculi open into them. In sections made by grinding, these canals, and also the lacunae and the canaliculi, become filled with debris and appear black by transmitted light (Fig. 9.4). Similar channels, known as *Volkmann's canals*, pierce the bone from the outer and inner surfaces and communicate with the Haversian canals, so that the bone possesses a com plete system of channels containing blood vessels and nerves.

In *lamellar compact bone* the concentrically-arranged lamellae form the *Haversian systems* or *osteones*, the intervals between being filled with irregularly arranged interstitial lamellae. These latter represent the partially remodelled remains of previous osteones. At the periphery of each osteone is a narrow fibre-free band known as a cement line. Immediately beneath the bounding periosteum, and also lining the marrow cavity, is found a layer of circumferential lamellae running parallel with the surface, thus enclosing all the osteones (see Fig. 9.3). The bone cells lie in their lacunae, between or within the lamellae, and also line the Haversian canals. The canaliculi anastomose freely, and some of those belonging to the innermost and outermost layers open respectively on to the endosteal and periosteal surfaces: some also open into the Haversian and Volkmann's canals.

The fine collagen fibres of the lamellae run parallel with one another within any one lamella, but the direction varies in different lamellae. The arrangement of these fibres is determined by the various tensions and stresses which the bone has to meet but it is predominantly spiral. In addition to these fine fibres, the outer parts of the bone are perforated by coarser fibres, the *perforating fibres of Sharpey*, which are continuous with the periosteum: these are most numerous in the regions of attachment of ligaments and tendons, and serve to strengthen their insertion.

In woven bone the lamellae are absent, and there are no osteones.

The Haversian and Volkmann's canals contain one or more blood vessels, nerves and connective tissue: they are lined by bone cells and pierced by the openings of numerous canaliculi which belong to the lacunar system.

The Cells of Bone

(*a*) **Osteoblasts and Osteocytes** (Figs. 9.2, 9.6, 9.7). Osteoblasts are the cells which secrete the bone matrix. They are typically of an ovoid shape, with slender cytoplasmic processes extending from them into contact with those of adjacent cells. They have a basiphilic cytoplasm due to the presence of large amounts of rough-surfaced endoplasmic reticulum with its associated ribosomes; there is a prominent ovoid nucleus and a well-developed Golgi apparatus.

Osteoblasts are always found on the growing surfaces of new bone, where the matrix is being laid down and where calcification is taking place, although their role in this latter process is still not understood.

As the osteoblast secretes the bone matrix it often becomes completely surrounded by the substance which it has itself secreted, and thus becomes trapped; it is then termed an osteocyte and occupies a space or *lacuna* in the matrix (Fig. 9.6). As the osteoblast had numerous slender cytoplasmic processes, small *canaliculi* extend from each lacuna out into the matrix which has been deposited around them. In life it is likely that short processes from the osteocyte extend into the canaliculi. The osteocyte is a cell which is not actively synthesizing, so that its cytoplasm lacks the typical basiphilia and associated ultrastructure of the osteoblast.

If an osteocyte is subsequently freed from its lacuna by absorption of bone during the process of a remodelling of the form of the bone, then it is possible that it may revert to the actively synthetic osteoblastic form or it may become incorporated with other similar cells to give rise to a multi-nucleate osteoclast.

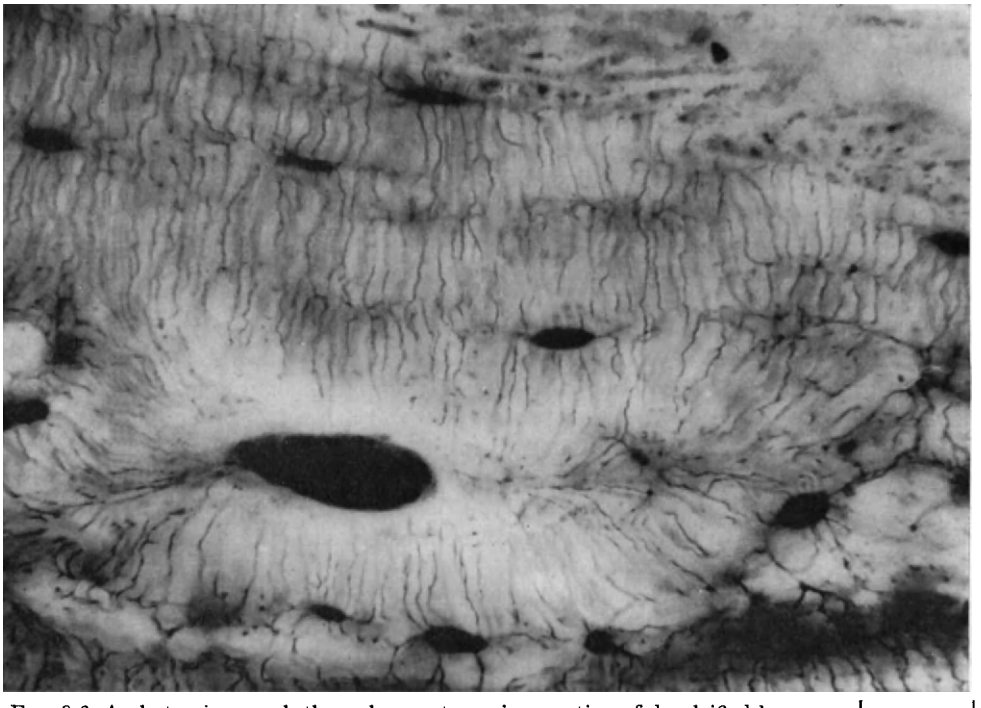
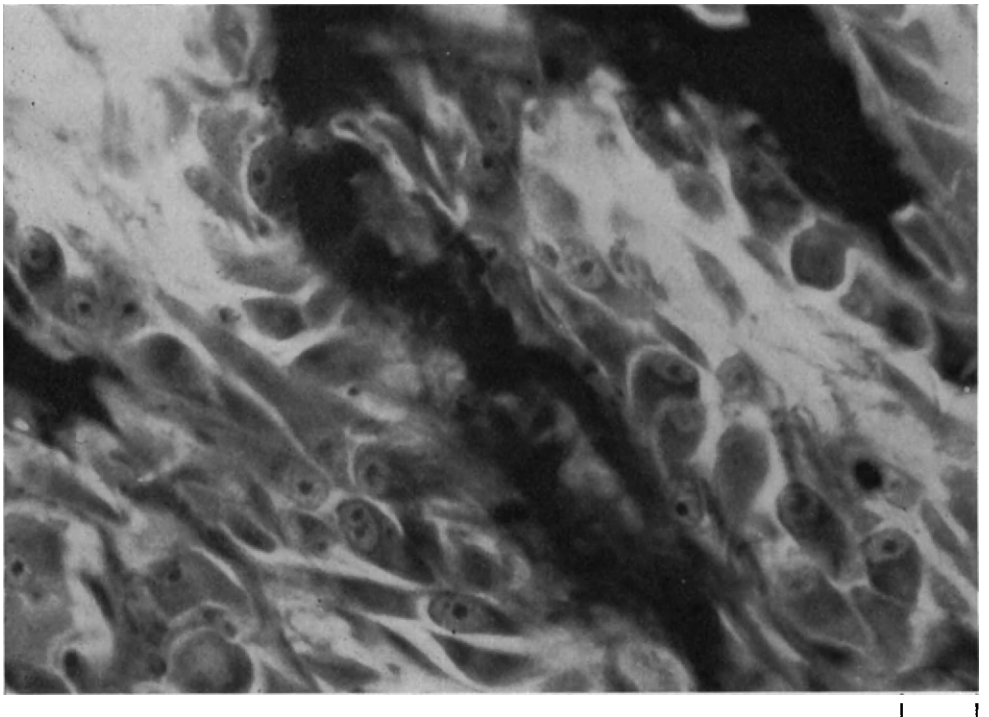


FIG. 9.6. A photomicrograph through an osteone in a section of decalcified bone. The cells and their processes have been stained and appear dark.

10 μm.



10 μm.

FIG. 9.7. Two developing spicules of bone, stained dark, run diagonally across the picture. They are surrounded by spindle-shaped osteoblasts. The nucleoli and darkly staining cytoplasm indicate the high degree of synthetic activity taking place in these cells.

(*b*) **Osteoclasts** (Fig. 9.8). The osteoclast is a cell which is responsible for the resorption of bone which occurs during the growth and remodelling of the skeleton. The osteoclast is a large (over 100 μm. in length) cell with numerous nuclei; in any one cell these may be twenty or more in number. Osteoclasts are invariably associated with a bony surface, just like osteoblasts, but with a surface which is being actively destroyed, not formed. In many cases the osteoclasts appear to be embedded in a pit in the bone matrix (in what is often termed a *Howship's lacuna*).

The cytoplasm of the osteoclast has an acidophil staining reaction and often has a vacuolated appearance and it may show a marked reaction to the test for the presence of acid phosphatase which characterizes lysosomes. Where the osteoclast is in contact with bone, the cytoplasmic border of the cell shows specialization into what is called a "brush" or "ruffled" border. The E/M shows that this is in reality due to the presence of large numbers of pseudopodial-like

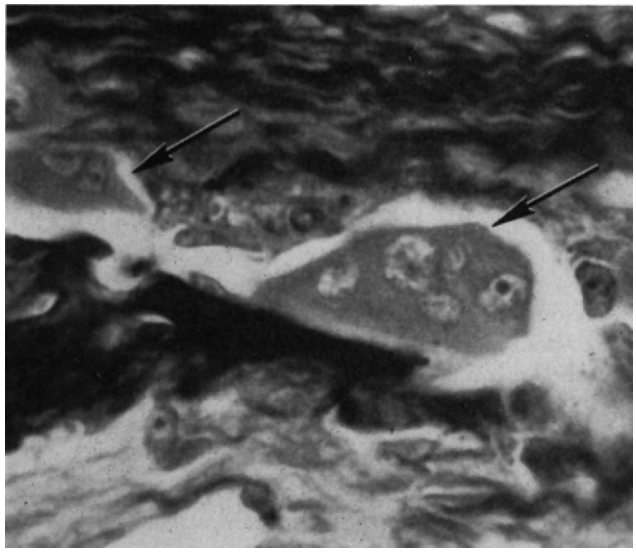


FIG. 9.8. This micrograph shows two osteoclasts (arrowed) at a site of bone resportion. The cell on the right of the picture shows five nuclei and a clear "ruffled" border where it is in contact with the bone. The space surrounding the cells is an artifact due to shrinkage of the tissue during processing. 10 μm.

folds of cytoplasm with many membrane-bound vesicles in the adjacent cytoplasm. Exactly how the osteoclast erodes bone is not yet quite clear, but it seems highly likely that the secretion of proteolytic enzymes may be involved in some part of this process, followed by pinocytosis of the degraded material by the osteoclast.

Blood Vessels, Lymphatics and Nerves of Bone

(*a*) **Blood Vessels.** A long bone is supplied by a medullary artery that enters by means of the medullary or nutrient canal near the centre of the shaft. As it pierces the compact bone this artery sends branches to the vessels in the Haversian canals, and on reaching the central marrow cavity it divides into an ascending and a descending branch which supply all the marrow, breaking up into a capillary network.

In addition the periosteum brings blood vessels into the bone. The Volkmann's canals everywhere pierce the bone carrying into it small blood vessels: these either run in the Haversian canals of compact bone or form a network in the marrow spaces of spongy bone. From the

Haversian canals the vessels send branches into the marrow cavity and their capillaries anastomose with those derived from the other group of arteries.

The capillaries are collected into thin walled veins that have no valves: these pass out by the Haversian canals, ultimately reaching the periosteum again either through the Volkmann's canals or through the medullary canal.

(*b*) **Lymphatics.** There is difference of opinion as to whether lymphatics occur in compact bone; some authors, however, describe them in the periosteum.

(*c*) **Nerves.** Nerve fibres, both myelinated and non-myelinated, are found accompanying the blood vessels in the canals; they have been found in the periosteum, but nerve endings in the osseous tissue are very difficult to demonstrate. Myelinated nerves are numerous in the marrow.

Periosteum and Endosteum

The periosteum invests all bones except at joint surfaces, it can easily be stripped off from young bones, but is more firmly adherent to older bones. For this reason it is easy to break a young bone without tearing the periosteum: such a break is called a green-stick fracture. The periosteum is particularly firmly attached when covering short bones, epiphyses, or the places of attachment of tendons, its collagen fibres being continued directly into the bone as *Sharpey's fibres.* The periosteum consists of two layers, the outer being dense collagenous connective tissue with blood vessels and nerves and containing only a few fibroblasts, while the inner is more loosely arranged, less vascular and provides the Sharpey fibres. This inner layer is very cellular in young bones and contains fusiform osteogenic cells and, next to the bone, osteoblasts (see p. 103). There is reason to believe that the osteogenic cells (which are not so flattened as the fibroblasts of the outer layer) give rise to the osteoblasts or bone-forming cells. The inner layer of the periosteum is thus known as the *osteogenic* layer. The blood vessels pass from the outer layer through the inner one to reach Volkmann's canals and eventually the Haversian canals.

The periosteum is of great importance. It is concerned with the nourishment of the bone by bringing to it the blood vessels and nerves, and it also serves to give attachment to muscles and tendons. In the normal adult the osteogenic activity of the periosteum is dormant, but in response to trauma and certain other pathological conditions, the power of bone formation returns, the deep layer of cells multiplying to produce osteoblasts. Fragments of periosteum transplanted elsewhere in the organism may also show this property of forming bone. During normal bone formation and during repair the periosteum also serves to limit the extent of the bony transformation.

A corresponding, but less marked layer on the internal surfaces of the bone, i.e. lining the marrow cavity, is termed the *endosteum.*

DEVELOPMENT AND GROWTH OF BONE

Some bones are developed directly from the embryonic mesenchyme: this process is called intra-membranous ossification, and is the way in which most of the bones of the face and cranial vault and the clavicles are formed. The other bones are first of all modelled in the form of cartilage, which is subsequently destroyed and replaced by bony tissue: this is called endochondral ossification. It must be emphasized, however, that the actual histogenesis of the osseous tissue is in each case exactly the same, and consists in the transformation of embryonic connective tissue into bone by the activity of the osteoblasts. Cartilage is never changed into bone: it is progressively but completely destroyed and replaced by tissue (*osteoid*) that is quickly calcified: if the calcium to phosphorus ratio in the blood is disturbed calcification is interfered with, and there is an excessive formation of osteoid tissue, as in rickets. The juvenile bones are merely temporary structures, which are necessary during development to provide a solid framework for the growing body. The final architecture of the true bone in the

body is determined by the optimal reaction to the various stresses and strains imposed on the different structures, and is not completed until after puberty: cartilage provides a temporary framework that can grow and that is gradually replaced by (but is never changed into) true bone.

A. Intra-membranous Ossification

In those places where bone formation occurs without a temporary scaffolding of cartilage the primitive connective tissue becomes richly vascularized, and the cells, most of which are stellate, multiply by mitosis: the interstitial matrix at this stage contains bundles of fine collagen fibrils. Very shortly bands of differently-staining material appear between the cells: this

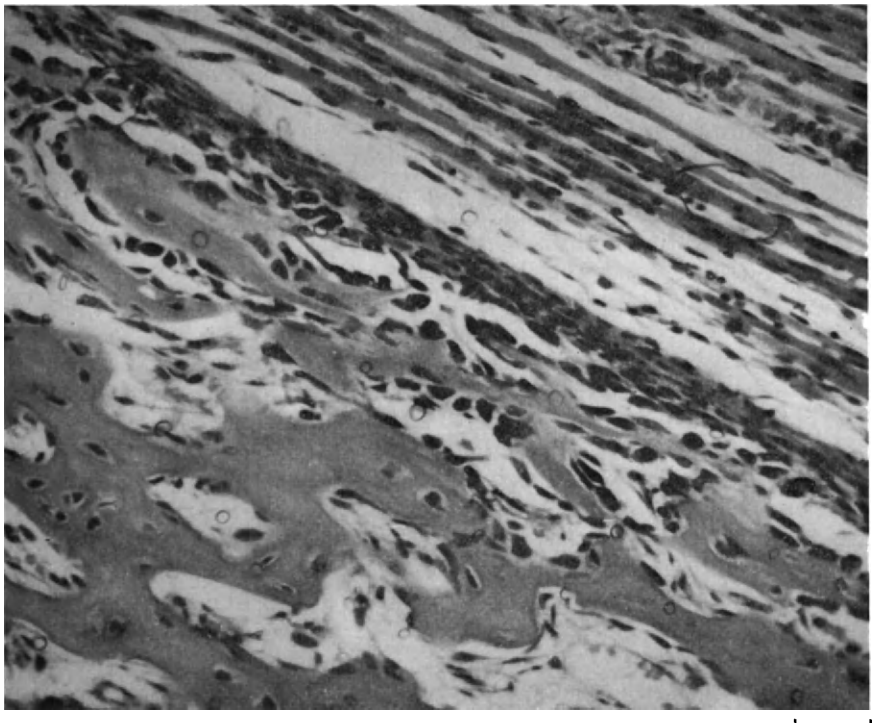


FIG. 9.9. Developing membrane bone; the bone spicules are seen to be covered with a layer of darkly staining osteoblasts, some of which may be seen enclosed within the deeper spicules of bone (bottom left) to become osteocytes. Developing striated muscle occupies the upper part of the micrograph, separated from the bone by the dense periosteum. 50 μm.

new interstitial substance replaces the original matrix, and ultimately becomes calcified. At the same time the cells increase in size, become polyhedral, and are found lying along the surface of the bands of newly formed interstitial matrix which constitute the developing trabeculae of the membrane bone (Figs. 9.7, 9.9). The cells become basiphilic in their cytoplasmic staining reaction and are transformed into typical osteoblasts. Since they do not multiply by cell division, their number is increased by further transformations of the osteogenic cells. When they revert to an inactive state they become flattened and spindle shaped.

The formation of the specialized interstitial matrix continues at the periphery of the original mass due to the activity of the osteoblasts and soon calcification of the interstitial matrix begins with deposition of small crystals of apatite. As new matrix is laid down, the osteoblast layer moves out with it on its outer surface and at the same time some osteoblasts

become included as the matrix forms round them: these become the isolated osteocytes (see Fig. 9.9). The original cells were connected to one another by their processes, and the osteocytes derived from them are also star-shaped with processes penetrating into the canaliculi and connecting the cells together.

The surrounding connective tissue becomes differentiated into the closely adherent periosteum, and the osteoblast layer in this way comes to lie between it and the growing bone.

Thus irregular trabeculae of woven bone are formed: they anastomose and enclose the primary marrow spaces. As growth proceeds these spaces are gradually narrowed down into the channels which carry the blood vessels and nerves in compact bone, and into the marrow spaces in spongy bone. The woven bone which is formed initially in intra-membranous sites does not have lamellae with the collagen fibrils arranged in a regular pattern. This lamellation only appears when secondary bone is subsequently formed by remodelling.

In the case of the skull bones, the spongy bone originally formed is rapidly absorbed on its inner surface by the action of the osteoclasts while simultaneously new bone is laid down by the subperiosteal osteoblasts: this is to provide for the rapid growth in the size of the skull that is required for the growing brain.

B. Endochondral Ossification

This type of ossification occurs in hyaline cartilage, which degenerates to be replaced completely by bone. Briefly, the process is as follows. (See Fig. 9.10.) A *primary ossification centre* appears near the middle of the shaft of a cartilaginous model of a limb bone; here the cartilage cells become much swollen and the matrix between the lacunae becomes reduced to little more than thin plates or septae. If there are adequate concentrations of calcium and phosphate ions in the tissue then these remains of the cartilaginous matrix calcify. At the same time a layer of bone is laid down immediately beneath the investing perichondrium (which may now be termed the periosteum) by the activity of cells in its deeper layers. This bone provides a rigid mass (known as *subperiosteal bone*) surrounding the calcified cartilage like a collar; it provides a valuable means of support in the later stages of ossification (see Figs. 9.11, 9.13). Vascular periosteal tissue now invades the calcified cartilage matrix, eroding the matrix and filling the cavities so formed (Fig. 9.14). Some of the cells which are brought in by this proliferation become differentiated into osteoblasts, whilst others form the haemopoetic elements of the marrow. The process of ossification proceeds and extends from the centre of the shaft towards the ends or *epiphyses*, the newly formed bone being deposited on the framework formed by the persistent spicules of the cartilage model. At this stage the epiphyses still consist entirely of hyaline cartilage. At a later date a *secondary ossification centre* appears in each epiphysis, periosteal tissue grows in and the cartilage is removed and bone laid down as before. Ossification from this centre in the epiphysis extends outwards. The result of these changes is that the growing bone comes to consist of a bony shaft (the *diaphysis*) separated from its ossifying epiphyses by a layer of cartilage (the epiphyseal plate) (Fig. 9.12), while the articular surfaces of the epiphyses are also covered by cartilage. Growth in the length of the bone depends entirely on these epiphyseal plates, which persist until skeletal maturity. The times of this differ widely between individuals, between the sexes and between the individual bones of the skeleton; a knowledge of them is important in orthopaedic and forsenic medicine. Eventually, when growth is complete, the epiphyseal cartilage plates become resorbed and replaced by bony trabeculae ("closure" or fusion of the epiphyses).

Once the epiphyseal bone has joined the shaft bone, then further growth in length is impossible. If there is overactivity of the adenohypophysis with consequent excess production of somatotrophic hormone, then excess growth of the immature bones results in a general increase in the stature of the bony skeleton—a condition known as *gigantism*. If excess of somatotrophic hormone is produced after the fusion of all the epiphyses has taken place then

an overgrowth, confined to certain bones of the face, hands and feet occurs. This is the condition of *acromegaly*.

The cells of the epiphyseal cartilage plates are arranged in characteristic columns (Fig. 9.12). Proliferation of these cells occurs at the epiphyseal end of the cell columns at a rate which

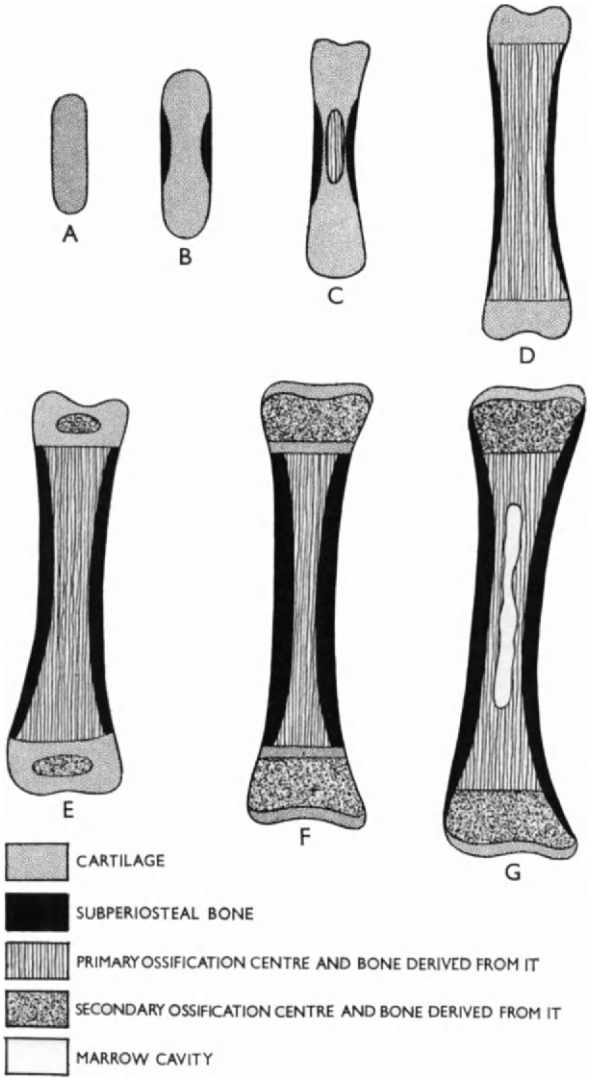


FIG. 9.10. A diagram to show the various stages in the endochondral ossification of a long bone.

is balanced by their degeneration and removal by the development of new bone at the shaft end. Increase in length of the bone is therefore a "growing away" of the cartilaginous epiphyseal plate from the site of ossification and bone formation on its shaft side. As a result of this the plate remains approximately the same thickness until growth is completed. Growth in thickness of the walls of the shaft is due to the progressive deposition of new bone in a subperiosteal position without any preliminary cartilaginous scaffolding being laid down.

Osteones are formed only late in bony development. Foetal bony trabeculae which consist of woven compact bone are ultimately absorbed and they are replaced by secondary compact bone which is arranged as osteones with their concentric bony lamellae. At the same time a large part of the trabecular bone in the centre of the shaft of a limb bone is resorbed with the formation of a large central marrow cavity.

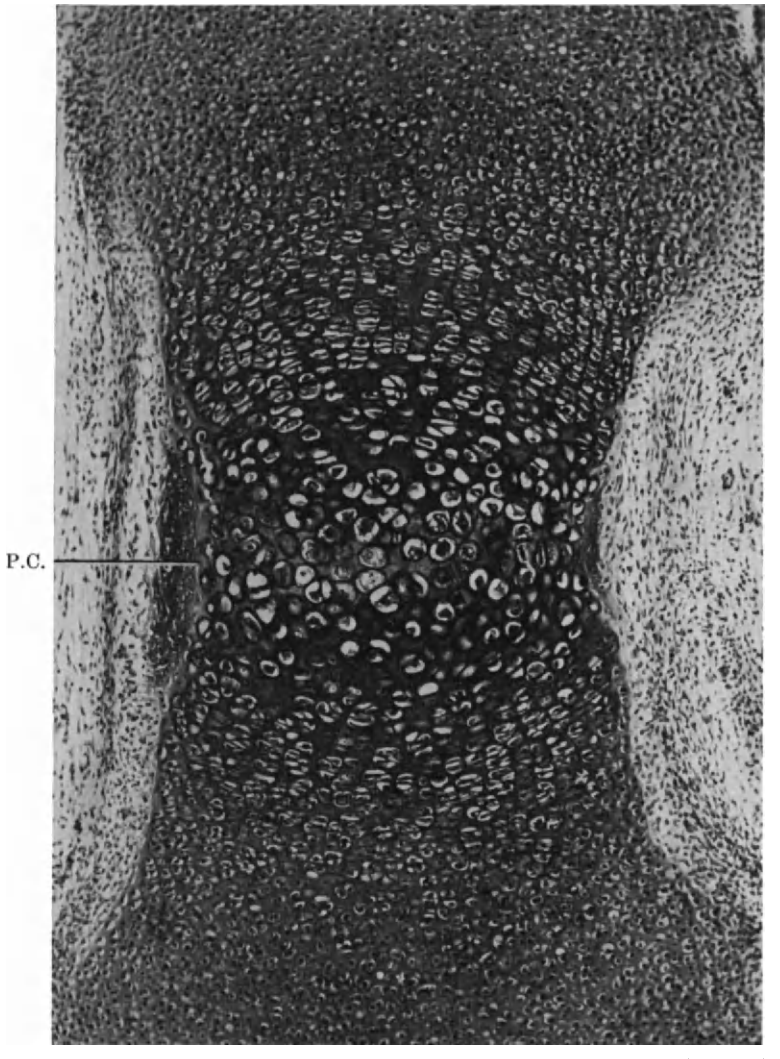


FIG. 9.11. The primary centre of ossification in a long bone of a 10 week human foetus. The subperiosteal collar of bone is indicated at P.C. This corresponds to stage B, Fig. 9.10.

Details of the histological changes involved in endochondral ossification

These can be seen by examining a longitudinal section of developing long bone that passes from the epiphyseal cartilage to the centre of the shaft.

The greater portion of the epiphysis consists of ordinary hyaline cartilage; as this grows in its usual way the cells have the typical arrangement in groups. Towards the ossifying shaft the cells are seen to be arranged in columns orientated along the direction of the long axis of

the bone (Fig. 9.12): they become flattened and are arranged like a pile of biscuits, separated from one another by a thin capsule only, while between adjacent columns are thick bands of the interstitial matrix.

Passing further towards the shaft the cells become larger and swollen, often containing vacuoles and a degenerating nucleus: this is the so-called "zone of hypertrophy". These cells

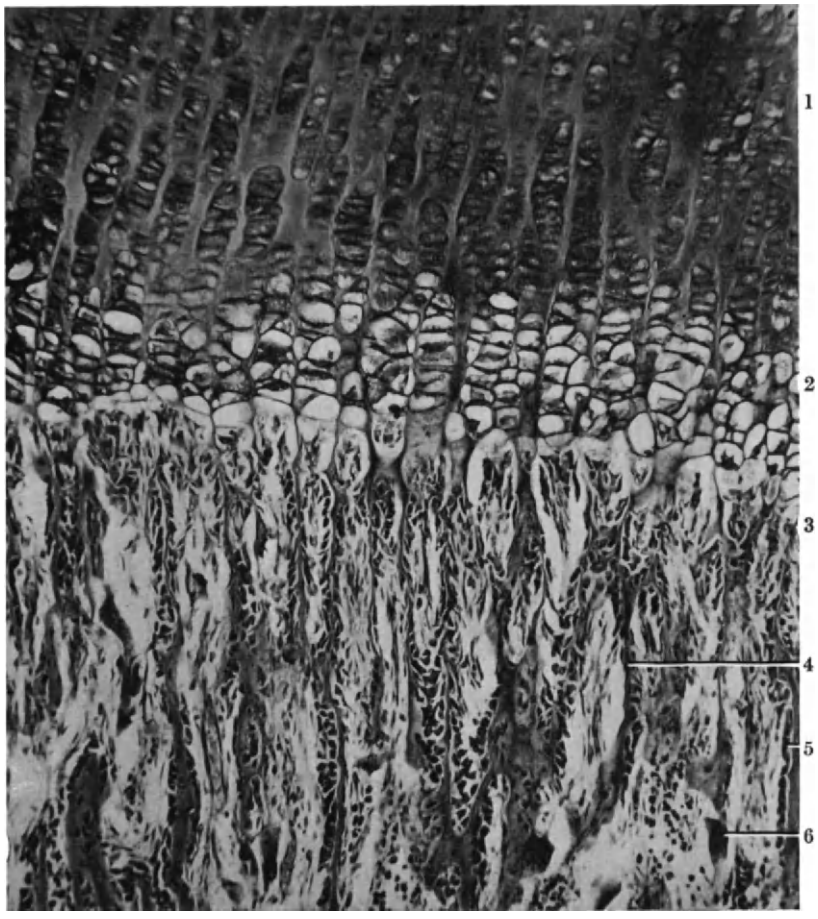


FIG. 9.12. A longitudinal section through the head of a developing long bone. 120 μm.

1. Layer of cartilage cell columns in the epiphyseal cartilage plate.
2. Zone of swollen cartilage cells (hypertrophic zone).
3. Zone of break-up of calcified cartilage matrix.
4. Trabeculae of calcified cartilage covered with osteoblasts laying down bone.
5. Layer of osteoblasts.
6. Osteoclast.

are rich in glycogen. In this region also the interstitial matrix is becoming calcified, and consequently stains more deeply than in the hyaline cartilage. Still a little nearer the centre of the shaft the cells are found to be shrunken and degenerated.

While these changes are occurring a collar of bone is being laid down as in the ossification of a membrane bone immediately beneath the perichondrium or periosteum surrounding the centre of the shaft. Vascular periosteal tissue (sometimes called the "irruptive periosteal bud") then penetrates through this into the region of calcified cartilage and this results in

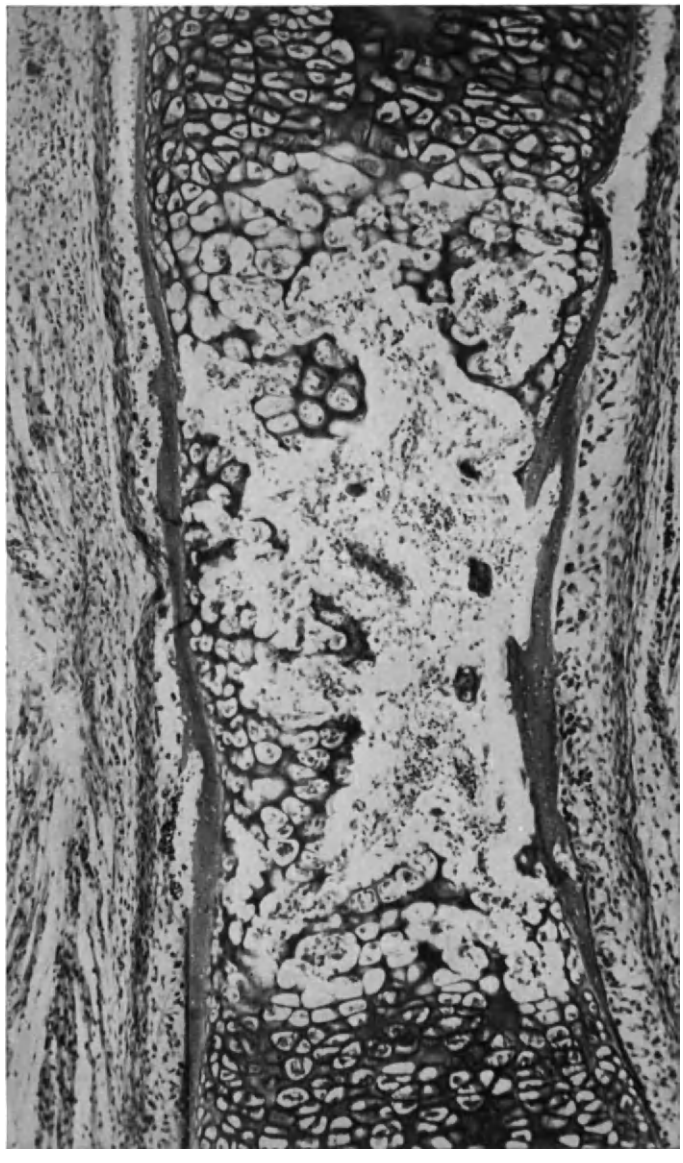


FIG. 9.13. Longitudinal section through the shaft of a long bone in a 10 week human foetus. This shows how extensive the resorption may be during the process of ossification and hence the need for the support provided by the subperiosteal collar of bone. 100 μm.

chondrolysis or cartilage resorption, with osteoclasts, the vascular endothelium and connective tissue cells all apparently involved in this process. As the cartilage is absorbed, large spaces are formed by the opening up of the thin cartilage capsules, and these spaces are filled with the irrupting blood vessels and embryonic connective tissue: this is seen in the zone labelled 3 in Fig. 9.12 and also in Fig. 9.14. Here the spaces between the remaining bars of calcified cartilage matrix contain large numbers of stellate embryonic connective tissue cells, with their processes in contact and actively multiplying: they are packed closely together with

blood capillaries and some large multinucleated osteoclasts. This tissue is the embryonic marrow. As one moves still further towards the centre of the shaft of the bone it is possible to see that where the embryonic marrow cells come to touch the calcified walls of the excavated spaces they are transformed into osteoblasts and arrange themselves as a layer along the calcified cartilage bars. These osteoblasts then lay down osteoid tissue, soft at first but soon calcified, as a thin layer covering the calcified cartilage remnants (Fig. 9.7). Layers of this tissue are laid down successively, some osteoblasts becoming imprisoned as bone cells. At first these bars of bone contain the remains of calcified cartilage within them, but later the

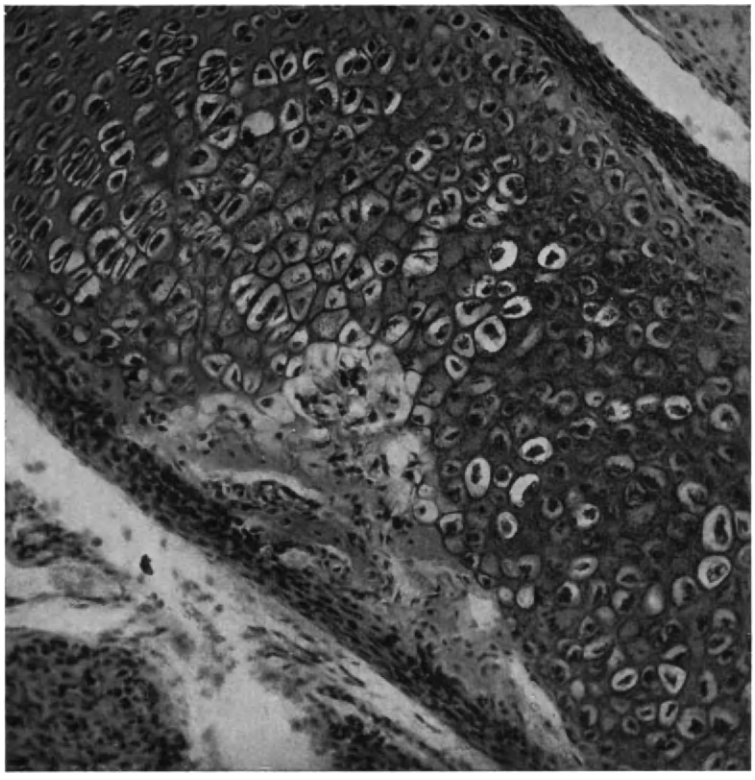


FIG. 9.14. A section of developing cartilage bone in the humerus of a human foetus which shows clearly the extensive vascularization of the primary ossification centre. This area of vascularization is sometimes called the "irruptive periosteal bud".

cartilage becomes resorbed. The embryonic bone marrow then becomes further differentiated with a preponderance of myeloid cells. Subsequently the trabecular bone formed by this process becomes thickened by the deposition of new bone concentrically around the trabeculae, so producing an appearance not unlike that of osteones. These, however, only develop after complete resorption of the primary woven compact bone has taken place and secondary bone has been laid down in the cavities so formed. Such internal reconstruction continues throughout life, osteoclastic activity alternating with that of osteoblasts. The partly eroded remains of early osteones may be found persisting between the newer osteones as the *interstitial lamellae*.

Causes of Bone Formation

Bone formation, both normal and pathological, is stimulated firstly by local factors, e.g. tension, presence of various living or non-living inducing agents and secondly by hormonal factors.

The periosteum is of the greatest importance in bone formation: not only does it provide some of the blood vessels of bone, but it gives also the periosteal tissue and osteoblasts that grow in to give rise to the bone matrix.

The actual calcification may be due to the activity of a phosphatase, found in the region of the hypertrophied cartilage cells and in osteoblasts, but there is also evidence which suggests that the presence of alkaline phosphatase along with nucleic acids in the osteoblast cytoplasm is rather to be correlated with their own particular metabolic activities. The substrate conditions necessary for the deposition of calcium phosphate in the osteoid are not well understood. Calciferol or vitamin D is necessary for this calcification: in its absence there is overgrowth of improperly calcified osteoid tissue; this is known as *rickets* in children or *osteomalacia* in adults. There must also be a sufficient concentration of calcium and phosphate ions in the blood and tissue fluids, but other local factors may well be operative in calcification. It has been suggested, for example, that the cartilaginous matrix must be degraded by lysosomal activity in order to render it capable of being calcified.

Certain of the endocrine glands are concerned in bone formation, particularly the adenohypophysis (anterior lobe of the pituitary); in addition, any factor that disturbs the calcium and phosphate ionic balance interferes with bone production. The parathyroid hormone is concerned with this ionic balance, and when in excess causes a release of calcium and phosphate from the bony skeleton into the blood. These changes are accompanied by an increase in the numbers of osteoclasts. Calcitonin, secreted by the C cells of the thyroid, opposes parathyroid hormone and inhibits bone resorption with a lowering of the blood calcium; indeed it has been suggested that calcitonin may itself act as an osteogenic stimulus. The process is also dependent on the nervous system, probably through its control of the supplying blood vessels.

Disturbances of Bone Formation

(1) **Old Age.** The bones act as a storehouse for the body's calcium: in old age, when the excretion of calcium is diminished owing to diminished endocrine activity, calcium tends to be deposited in various tissues. Frequently the bones decrease in calcareous content and consequently become increasingly brittle. Recent work with the scanning E/M has shown that the brittleness of old bones may be attributed to an increase in the size of the crystals of apatite.

(2) **Atrophy.** Atrophy* may occur in old age, but it occurs commonly from disuse, because in the absence of the normal activity of associated muscles that is necessary for the building up processes constantly occurring in the bone, osteoclastic resorption becomes excessive; this is probably the cause of the atrophy that occurs after poliomyelitis and prolonged weightlessness, such as occurs in astronauts. Interference with the blood supply may also play a part in disuse atrophy. The actual bony substance may also be lessened due to an increase in the marrow as in certain blood diseases. In some cases there is an absorption of calcium salts leaving a soft tissue unabsorbed: this occurs in osteomalacia, and sometimes in rickets. In osteoporosis there is a thinning of the bony trabeculae and enlargement of the spaces due to lack of osteoblastic activity. These conditions may result from metabolic disturbances of dietary or of endocrine origin, particularly in connection with malfunction of the parathyroid or thyroid glands: they

* *Atrophy* refers to the wasting of organs and tissues, usually accompanied by retrograde changes and characterized by a diminution in size. It should be noted that the special functioning tissue may atrophy extensively without any corresponding diminution in the bulk of the organ. An actual shrinkage of individual cells is sometimes spoken of as *atrophy* proper, while a diminution in their number is referred to as *aplasia*.

frequently give rise to deformities because of the interference with the normal rigid framework of the body.

(3) **Hypertrophy.** True hypertrophy* of bone occurs consequent on increased activity of the attached muscles: this results in an increase in the bony ridges for their attachment. Disturbances in the remodelling of bone may occur as a result of dietary disturbance, such as the absence of vitamin A.

(4) **Repair after Fracture.** A fracture, unless of the green-stick variety (see p. 106), involves both a break of the bony tissue and a rupture of the closely adherent periosteum. After a break there is haemorrhage and formation of a blood clot, followed by an acute inflammatory reaction which results ultimately in the removal of the blood clot and of necrotic tissues. Dense connective tissue is then formed (which often becomes cartilaginous or fibrocartilaginous) so uniting the ends of the bones in the *fibrocartilage callus*.

The periosteal cells may proliferate and new blood vessels grow out into the clot from those already present carrying in vascular connective tissue with them. Very shortly some of the fibroblasts differentiate into osteoblasts, and trabeculae of bone appear: these are quickly calcified and this newly-formed woven cancellous bone constitutes the *bony callus*, which provides the union and support that is so urgently required. In the absence of periosteum, the bone cells can resorb the surrounding matrix, proliferate and set about active ossification in the clot, so that the callus is dovetailed into the spaces in the broken ends. One of the chief functions of the periosteum is to limit the new bone formation. The formation of callus is usually excessive, and later it is destroyed by osteoclasts and bone is rebuilt in the usual way, so that the permanently united bone has the same form that it had before the injury.

JOINTS AND SYNOVIAL MEMBRANES

Immovable bones, such as those of the skull, are joined together by a small amount of fibrous tissue between the apposed surfaces. If a joint permits of a small degree of movement, as between the vertebrae, the junction is effected by dense fibrous tissue and cartilage. When the bones are freely moveable the joint is said to be *synovial*. The articular surfaces are covered with a modified type of hyaline cartilage (see p. 89) and the cavity is enclosed by a fibrous capsule. This *joint capsule* consists of two layers, the external, usually composed of dense fibrous tissue (the capsular ligament), and the inner or *synovial layer*. This layer is best regarded as being composed of an intimal layer, lining the joint cavity, which rests on a layer of connective tissue or of adipose tissue usually termed the *subsynovial layer*. This latter merges with the fibrous tissue of the external capsular layer.

The subsynovial layer contains many blood vessels derived from a mass of capillary loops (the *circulus vasculosus*) near the margin of the articular cartilage, together with fibroblasts, a few macrophages, mast cells and leucocytes. This cell content varies between different joints. Where the synovial membrane is continuous with the articular cartilage, cartilage cells may be present in the subsynovial layer. The intimal cells of the synovial membrane are often described as epithelial or mesothelial, but they do not form a continuous layer as in typical epithelia or mesothelia; many discontinuities, often of up to 1 μm. are present in the intima. The E/M has shown that there is no basal lamina but that the intimal cells lie in or on an amorphous layer of material 0·4 μm. thick. The synovial membrane is often thrown up into synovial villi that project into the joint cavity. This is especially apparent where the synovial membrane rests upon adipose tissue as in the case of fat pads projecting into some of the joint cavities. Typical synovial membranes also occur in tendon sheaths and in bursae.

* It is usual to speak of *hypertrophy* as an enlargement of the individual cells, and of *hyperplasia* as an increase in the number of the cells. Actually both processes are usually present together. Some workers prefer to apply the term *hypertrophy* to a disproportionate increase of an organ or tissue or some of its essential component elements to meet a demand for increased functional activity, either physiological or abnormal, and to define *hyperplasia* as the proliferative changes (not of the nature of an actual tumour growth) that occur where no useful purpose is served by such proliferation.

The synovial fluid that moistens the surfaces is present only in very small amount; it is basically a transudate of blood which is modified by the cells of the intimal layer of the synovial membrane by the addition of hyaluronic acid. This fluid helps to nourish the articular cartilages and is of great importance in the lubrication of the bearing surfaces of the joint. Current opinion is that the hyaluronic acid is added to the synovial fluid by the action of the intimal cells, although Asboe-Hansen believes that it is produced by the mast cells which are found in the subsynovial layer. The synovial membrane is also important because of its content of macrophages, which serve to remove from the joint cavity the detritus formed by wear and tear of the articular cartilage.

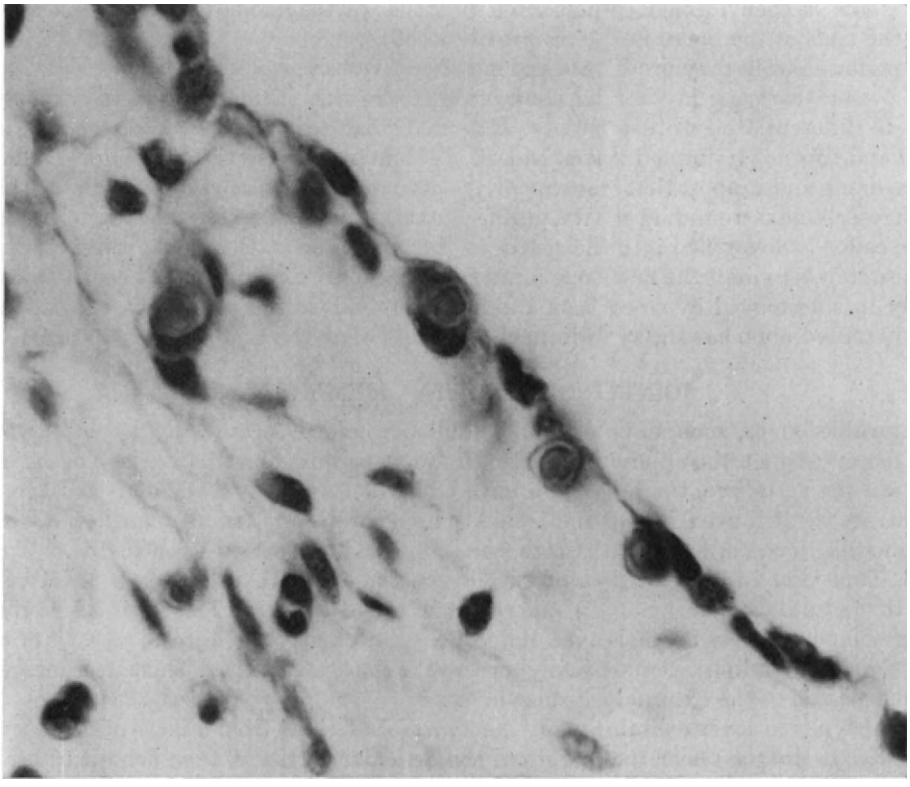


FIG. 9.15. A photomicrograph of the synovial membrane in a joint cavity of a very late human foetus; the actual cavity is to the right of the illustration. Note the thin intimal layer lying upon the loose subsynovial layer. 15 μm.

The blood vessels are found in the looser part of the connective tissue and also in the larger of the folds. There is a rich lymphatic supply in the form of two plexuses, but no lymphatics are found in the synovial villi. Nerve fibres, mostly non-myelinated, run with the blood vessels, and end in free ramifications, in bulbs or in Pacinian corpuscles: the great number of these sensory endings in the joint capsules and ligaments accounts for the extreme pain of arthritis. The nerve fibres are chiefly sympathetic in the joint capsule, but largely somatic in the synovial membrane where they form a nerve plexus.

In cases of injury the synovial membranes show the usual inflammatory reaction, and the amount of fluid in the joint cavity may be increased, due in part to an increase of synovial fluid and in part to inflammatory exudation. Regeneration of the synovial membrane can take place extremely rapidly.

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CHAPTER 10

MUSCULAR TISSUE

Muscular tissue is responsible for the movements of the body and for the movements of its various parts with respect to one another; it carries out mechanical work by contracting, which involves a shortening and thickening of its fibres. It is made up of cells or fibres, elongated in the direction of contraction, that contain contractile fibrils (myofibrils): the fibrils lie in the undifferentiated cytoplasm (sarcoplasm). Sometimes the fibres appear homogeneous as in smooth muscle: sometimes they consist of alternating bands of material, which produce the dark and light striped appearance of striated and cardiac muscle when stained with iron haematoxylin or studied with polarized light. Usually, smooth muscles are independent of voluntary control: striated muscle contracts under voluntary control, and cardiac muscle although striated contracts automatically.

(1) SMOOTH MUSCLE

This type of muscle is characterized by a slow and sustained mode of contraction. It is independent of voluntary control (excepting, perhaps, the ciliary muscle of the eye by which the curvature of the lens is changed for focusing at various distances) and its tone is maintained after section of its nerve supply.

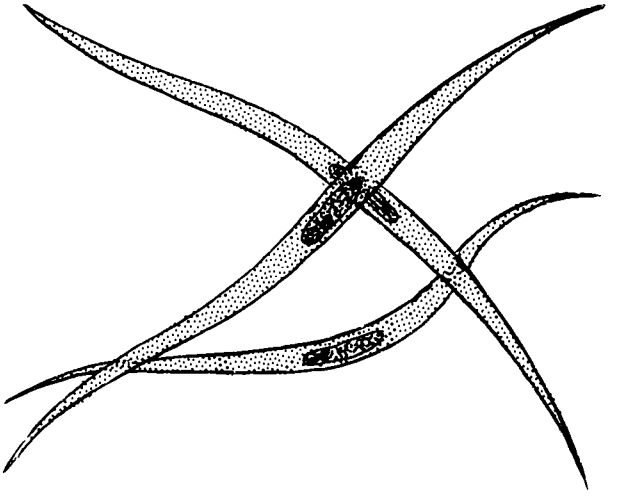

FIG. 10.1. A diagram of the typical form of dissociated smooth muscle cells as seen in a microscopical preparation.

Smooth muscle consists of long spindle-shaped cells (Fig. 10.1), round or angular in transverse section, and containing an ovoid central nucleus. The length of the cells varies enormously: they may be as short as 20 μm., while in the gravid uterus they may exceed 500 μm. in length. The sarcoplasm contains fine longitudinal myofilaments but no cross striations are visible with the optical microscope. Each fibre is bounded by a delicate plasmalemma. The arrangement of the fibres in the muscle as a whole varies; they may be grouped in dense sheets or layers, as in the alimentary canal (Fig. 10.2), form a network as in blood vessel walls, or even lie scattered as single fibres or small isolated groups as in the skin.

Smooth muscle cells may form a single circumferential sheet in the walls of hollow viscera; contraction of the muscle fibres then results in the constriction of the lumen of the viscus and an increase in the pressure of any contents. If, as in the gut, the muscle fibres in the wall form two sheets with their fibre axes arranged at right angles, then more complex movements may result such as the churning movements or regular peristaltic waves which are found during digestion. Contraction of the circular muscle forms a narrow ring of constriction which decreases the diameter of the lumen. Simultaneous contraction of the longitudinal muscle fibres causes a local shortening of that part of the tube above the constriction together with a

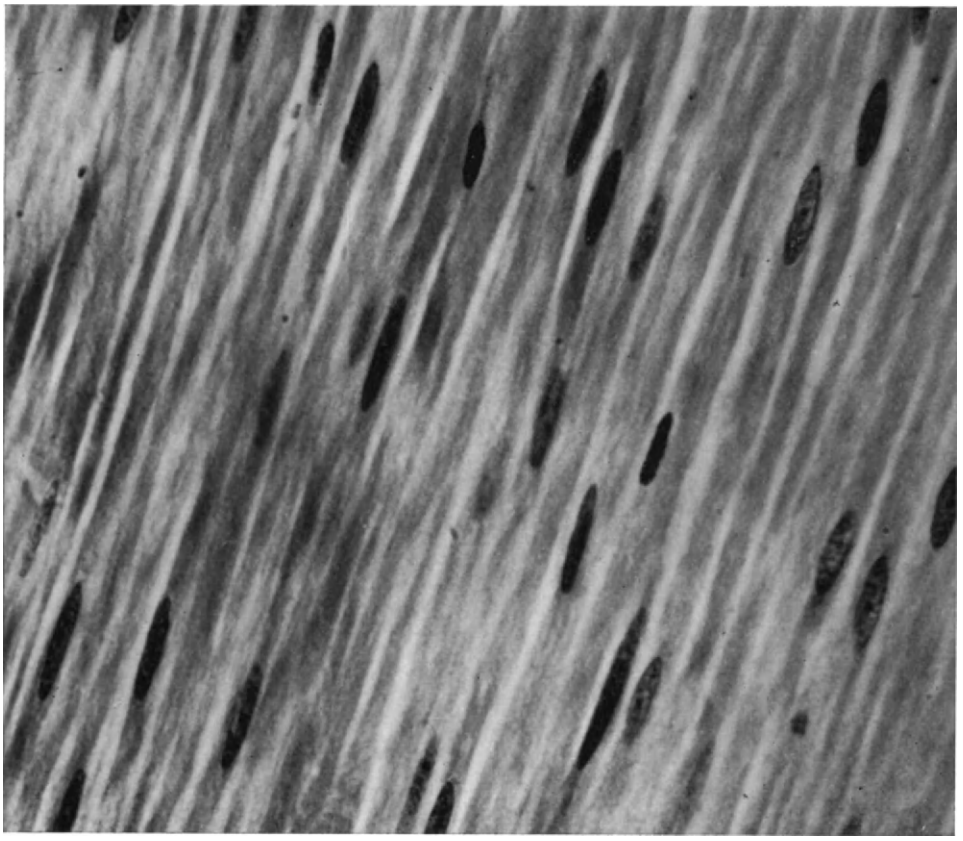

FIG. 10.2. Smooth muscle in the small intestine, seen when the cells are sectioned in a longitudinal plane. 20 μm.

local dilatation of the lumen immediately below, so that the contents are propelled along the tube.

The E/M shows that myofilaments occupy the bulk of the cytoplasm and are longitudinally orientated. Some authors (e.g. Lowy and Small) believe that myosin occurs in ribbon-like aggregates of molecules which are surrounded by the individual thin filaments of actin; others (e.g. Panner and his colleagues) suggest that all the longitudinal filaments in sections of smooth muscle are, in fact, actin. Although the fibrous proteins *actin* and *myosin* can both be isolated from smooth muscle fibres it is not yet clear what their exact relationship is to the longitudinally orientated myofilaments observed in these cells and further research is needed in this field. Mitochondria are clearly visible, often grouped around the nucleus.

FIG. 10.3(A). A low power electron micrograph of smooth muscle cells from the taeniae coli of the guinea pig. Parts of five cells (sectioned along their length) may be seen, one of which contains part of the cell nucleus. This latter cell shows vesicles of the Golgi apparatus and vesicles of the endoplasmic reticulum. 2 μm.

FIG. 10.3(B). Part of the cell membrane of two smooth muscle cells with a small amount of interstitial matrix between them. Notice the longitudinally arranged filaments in the cytoplasm and the characteristic pinocytotic vesicles at the cell surface. 0·5 μm.

Micrographs by Dr. H. Nisihara.

Numerous free ribosomes can also be seen, together with a Golgi apparatus usually located at one pole of the nucleus. There is a small amount of granular endoplasmic reticulum.

The individual muscle cells are covered with a coating which resembles the basal lamina of epithelia. In certain areas the membranes of two adjacent muscle cells come into close apposition—the so-called *nexus*; this almost certainly represents the site of low resistance pathways for the spread of excitation throughout the cellular sheet of muscle. The plasmalemma of the muscle cell is characteristically rich in pinocytotic vesicles or caveolae (Fig. 10.3). When packed together in a bundle the cells fit into one another so that the thick middle part of one cell is opposite the thin tapering ends of others: consequently in cross-section some of the fibres show a nucleus in the plane of section and some do not.

The connective tissue outside the muscle is continued into the spaces between the bundles, and contains the usual fibrocytes, macrophages, collagen and elastic fibres, blood vessels and nerves. Between the individual muscle cells is a delicate but dense network of fibres, partly collagenous or reticular and partly elastic, making a sheath for each cell which is continuous with the surrounding connective tissue. This arrangement is of great importance in the distribution of the force of the contraction of each muscle cell. The whole cell may contract throughout its length at one time, or a wave of contraction may pass over it, one part of the cell being contracted while the remainder is at rest.

Blood Vessels and Lymphatics

The blood supply is relatively scanty: the vessels run in the connective tissue between the bundles of cells mainly along the long axis of the muscle fibres but with occasional transverse anastomoses. Lymphatic vessels are also found in the connective tissue septa.

Nerve Supply

The nerve fibres supplying smooth muscle belong to the autonomic system. Their distribution is chiefly by a plexus with scattered nerve cells, the terminal fibres branching between the muscle cells and ending freely in close relation to them, sometimes actually appearing to be enclosed by the cells. Only a few of the cells appear to be in contact with a nerve ending and it is probable that the intercellular contact at the nexus is responsible for the spread of excitation from one cell to the next. Sensory endings are found in the interfascicular connective tissue.

Histogenesis

Smooth muscle is developed from the embryonic connective tissue: undifferentiated branching mesenchyme cells arrange themselves to form a network, the cells elongate, stretching out the nuclei, and the fibrils then appear. Some smooth muscle fibres appear to be formed from ectoderm (e.g. muscles of the iris).

Reaction to Various Conditions

(*a*) **Local Hypertrophy.** Hypertrophy of smooth muscle may occur, as for example in the uterine wall during pregnancy. Under such circumstances the individual cells enlarge enormously, and there may also be a small increase in the number of cells by direct transformation of connective tissue cells: after parturition the cells show fatty change and then decrease in size, while some may degenerate. The muscle in the walls of arteries subject to continuous high blood pressure may similarly show hypertrophy.

(*b*) **Injury.** Damage to smooth muscle is repaired chiefly by the activity of the connective tissue cells and by the formation of scar tissue. It has been suggested that to a very small extent there may be also a new formation of smooth muscle by mitotic division of those cells remaining. After death the muscle fibres contract.

Chief Distribution of Smooth Muscle

Walls of hollow viscera.
Walls of arteries and veins.
Walls of urinary and genital ducts.
Walls of gland ducts.
Walls of respiratory passages.
Arrectores pilorum muscles of skin.
Dartos muscle of the scrotum.
Muscles of the iris and ciliary body of the eye.

(2) STRIATED MUSCLE

Striated muscle tissue forms the substance of all muscles attached to the skeleton. The muscle fibres are under voluntary control, and when at rest the muscle is maintained in the slightly contracted condition known as "tone": if the nerve supply is cut this tone disappears. Contraction is a rapid process, and fatigue occurs quickly.

This type of muscle consists of cylindrical fibres that usually vary in diameter from 10 to 100 μm.; they may sometimes be as long as 130 mm. There is no relation between the size of a muscle and the dimensions of its component fibres; and, further, the fibres vary greatly in any one muscle. Striated muscle fibres are multinucleate.

The Whole Muscle (Fig. 10.4)

A striated muscle consists of many parallel striated muscle fibres united together in bundles (termed *fasciculi*) by connective tissue. The whole muscle is surrounded by dense connective

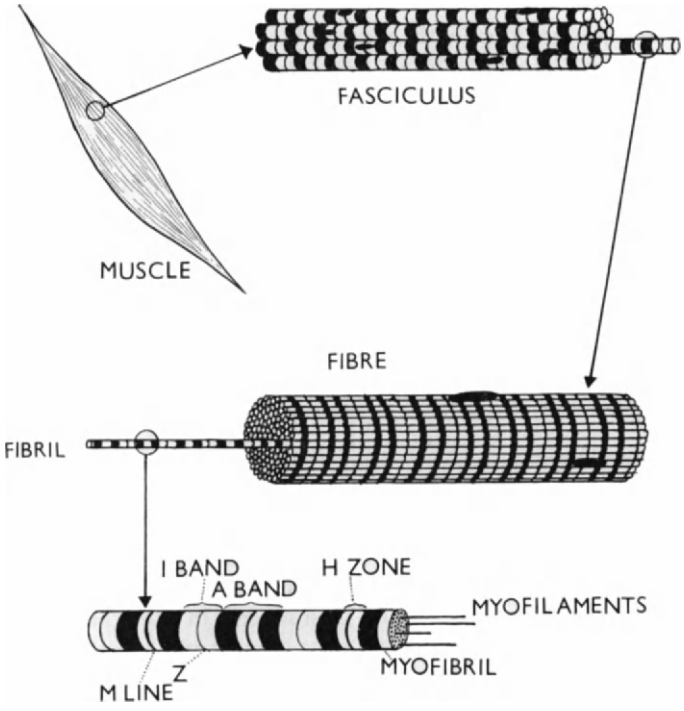

FIG. 10.4. A diagram of the levels of organization of striated muscle. The muscle is divided into fasciculi, which may be regarded as being composed in turn of fibres, myofibrils and myofilaments. The main regions of the sarcomere are indicated on the myofibril.

tissue, the *epimysium*: this sends trabeculae of looser connective tissue consisting of the usual fibres and cells into the muscle dividing the fibres into the fasciculi. The tissue ensheathing a fasciculus is sometimes called the *perimysium*, and from it pass delicate collagenous sheaths which enclose every individual muscle fibre and are known as the *endomysium*. This endomysium also contains fibrocytes, macrophages and fine reticular fibres: the macrophages play an active part if there is an inflammatory process in the muscle. Elastic fibres are also present in amounts that vary with the type of action of the muscle: they are particularly abundant in those muscles that are attached to soft parts, such as the tongue.

The fasciculi are arranged in definite patterns in each muscle. These patterns (e.g. whether the fasciculi are running in parallel, or are inserted at an angle into a tendon or into several connective tissue septa which then unite to form a tendon) are used by the anatomist to classify the striated skeletal muscles into several types, known as strap muscles, fusiform muscles, unipennate, bipennate and multipennate muscles.

The fascicular architecture of muscles bears a direct relationship to the contractile power of the muscle as a whole; with long, parallel fasciculi a large degree of shortening is possible but the pull is not very great. When the fasciculi are oblique and inserted into connective tissue septa (as, for example, in multipennate muscles) they are shorter and therefore the range of movement is correspondingly reduced. Many more fasciculi are packed into a given space, however, so that the total force which such a muscle is capable of exerting is greatly increased.

When a muscle fibre ends within a muscle the ending is tapered: when the fibres join a tendon they have rounded ends. At this junction of muscle and tendon the group of muscle fibres enclosed in perimysium continues as a tendon bundle, and the collagen fibres of the perimysium become directly continuous with the interfascicular tissue of the tendon: this arrangement provides a very rigid attachment.

Red and White Muscles

In most mammals all the muscles look red, in the frog the muscles are all white, while in some mammals and in birds some are red and some white. Microscopically two kinds of fibres can be distinguished, and in most mammals, including the human, all the muscles contain both kinds. The white fibre is poor in sarcoplasm, the fibrils are small and regular, and the nuclei are peripheral. The red fibres, on the other hand, contain much granular sarcoplasm, the longitudinal striation is very marked and the transverse striation irregular, and the nuclei are frequently central in position: the colour is due to the presence of myoglobin and cytochrome. The red fibres are particularly abundant in muscles that are in constant action.

The Microscopic Structure of Muscle (Figs. 10.4 to 10.12)

Each fibre consists of muscle cytoplasm surrounded by a very thin limiting membrane, the *sarcolemma*; this follows the shape of the muscle fibre in all its changes. The muscle cell cytoplasm is usually known as *sarcoplasm*, and in it are embedded the *myofibrils* that run parallel to one another throughout the length of the fibre. The fibrils may be closely packed together in the muscle fibre, or they may be grouped in bundles separated by sarcoplasm. In a cross-section of the fibre (Fig 10.6) the cut ends of the fibrils look like dots: if these fibrils are grouped together in columns then definite areas (*Cohnheim's areas*) can be seen in the section of the fibre, separated from one another by pale sarcoplasm. The myofibrils appear striated when seen under the optical microscope, because they possess differentiated regions which recur in a regular manner so giving the appearance of alternate light and dark segments. As the position of these bands in any one fibril corresponds with those in adjacent fibrils, the whole muscle fibre shows cross striations. This appearance is preserved in stained sections because the dark bands (the A bands) have a greater affinity for basic dyes than the light bands (the I bands). The A bands are so-called because they are birefringent in the polarizing microscope

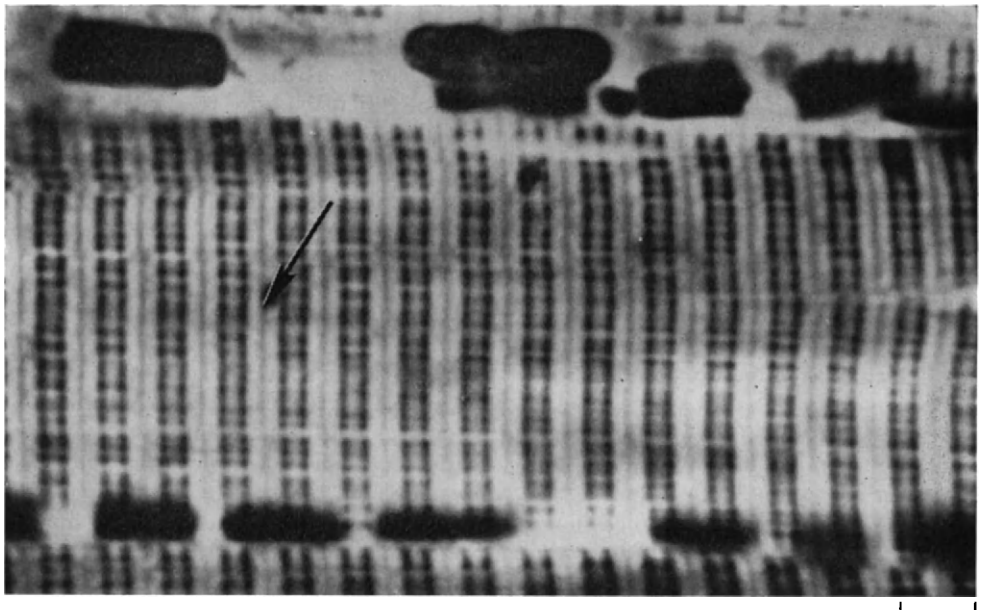

FIG. 10.5. A high power optical micrograph of striated muscle; this has been fixed in the extended condition. The two parts of the A band are stained dark and are separated by the clear area of the H zone. Some of the subsarcolemmal nuclei are clearly visible, together with the darkly staining Z band (arrow) bisecting the I band.

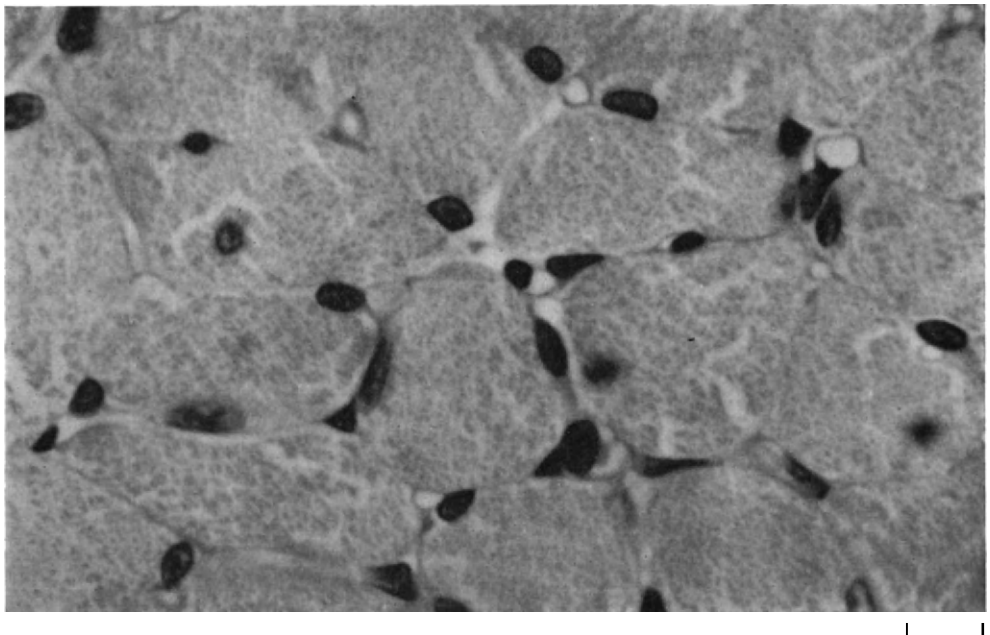

FIG. 10.6. Fibres of striated muscle seen in transverse section. Note the sub-sarcolemmal nuclei and the grouping of the myofibrils into the so-called "Cohnheim's areas".

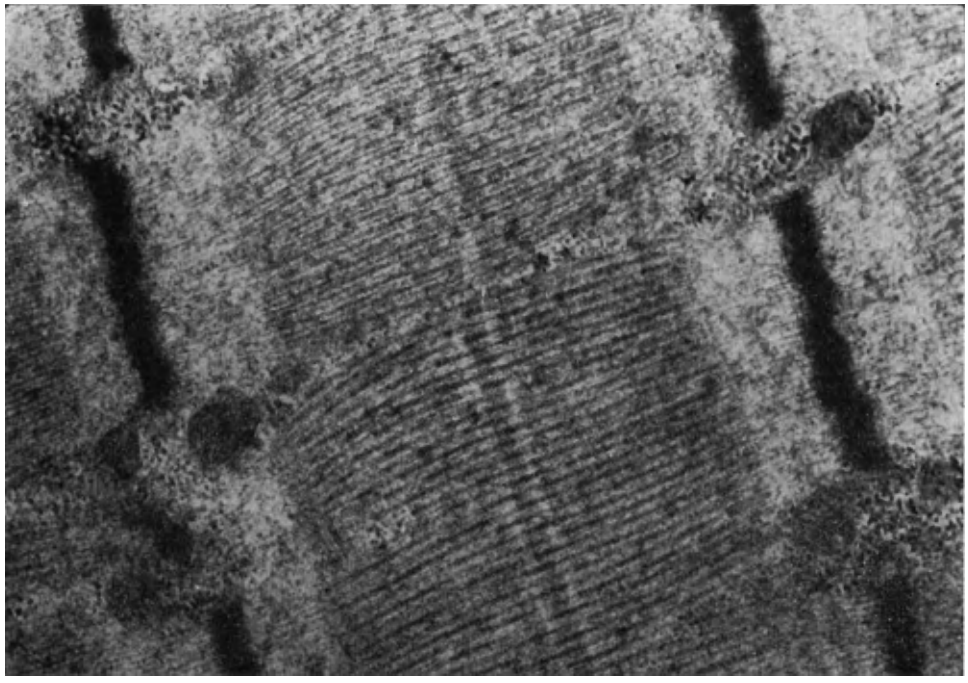

FIG. 10.7. An electron micrograph of a sarcomere of striated muscle seen in longitudinal section. The two dark lines are the Z bands. The A band, with its thick filaments of myosin, occupies the centre of the micrograph.

0·3 μm.

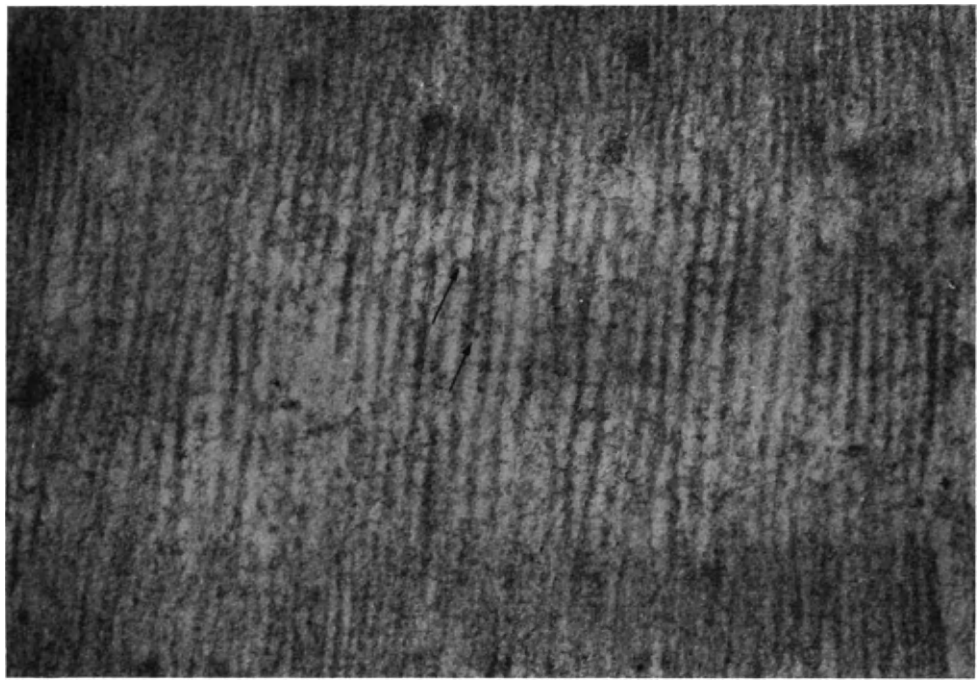

FIG. 10.8. A high power electron micrograph of striated muscle which has been preserved in glycerol at low temperature for some weeks before processing for the electron microscope. Myosin filaments of the A band are seen in the centre of the picture and some of the cross-bridges are indicated by the arrows.

0·15 μ

and are therefore *anisotropic*, whereas the I bands do not show birefringence and are consequently *isotropic*. There is a fine darkly-staining line (the Z band) running through the middle of the I band, whilst in a muscle fibre which is in the extended condition the A band is seen to contain a light area (the H band). These are shown clearly in Figs. 10.4 and 10.5.

Recent investigations with the E/M have shown that each myofibril consists of parallel arrays of smaller units—the *myofilaments*—which consist principally of the proteins actin and myosin. These are long molecules with their axes arranged parallel with that of the myofibril.

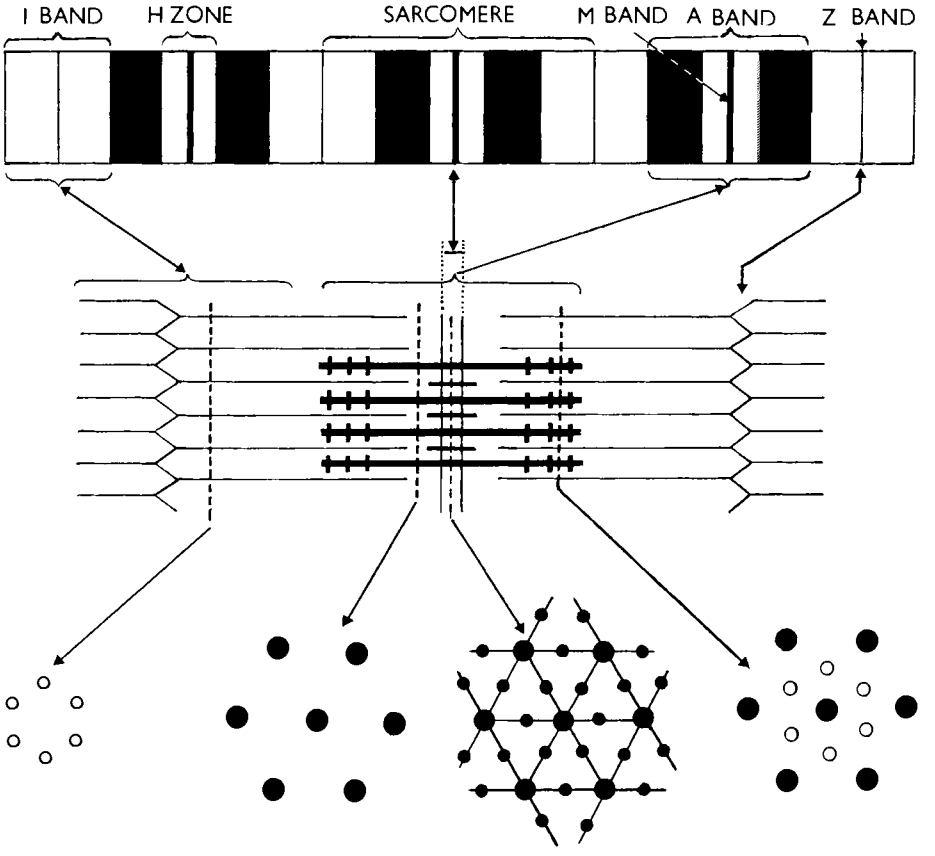

FIG. 10.9. A diagram of the electron microscopical organization of the sarcomere. The appearance in the optical microscope is indicated at the top of the illustration, whilst cross sections at the levels indicated by the dotted lines are at the bottom. From left to right these are through the I band, the H zone of the A band containing myosin only, the M band, the A band region which contains actin filaments in between the thicker myosin molecules.

Studies with negative staining techniques on isolated muscle proteins have shown that the actin is composed of an end-to-end aggregation of numerous globular subunits (the G-actin). Two of these aggregates twined around each other constitute the filament of F-actin (Fig. 10.10). The myosin is found to consist of much shorter, thicker rod-like molecules about 1500 Å in length with a short side projection at one end. These side projections are thought to constitute the so-called heavy meromyosin whilst the bulk of the long arm is light meromyosin. The heavy meromyosin portions probably form the cross bridges which are a feature of the E/M appearance of striated muscle (Fig. 10.8). The myosin myofilaments, about 1·5 μm. in length by 120 Å in diameter, are confined to the A bands of the muscle fibril, whilst the actin

forms the thin filaments about 2 μm. long by 80 A in diameter extending through the Z bands on either side towards the myosin molecules of the A band, with which they interdigitate (Figs. 10.7, 10.8, 10.9). If cross sections of myofibrils are examined with the E/M, the I bands will show only thin actin filaments arranged in a regular pattern (Fig. 10.9) whilst a section passing through the H band will contain thick myosin filaments only. When the muscle contracts the actin filaments slide in between the thick myosin myofilaments. This process seems to be mediated by a cyclical series of changes involving the heavy meromyosin cross bridges. One current theory proposes that these heads are contractile, due to a change from an extended polypeptide chain configuration to that of a contracted α-helix in the presence of calcium ions, which form a link between the meromyosin and the actin. Whilst these ions are present during

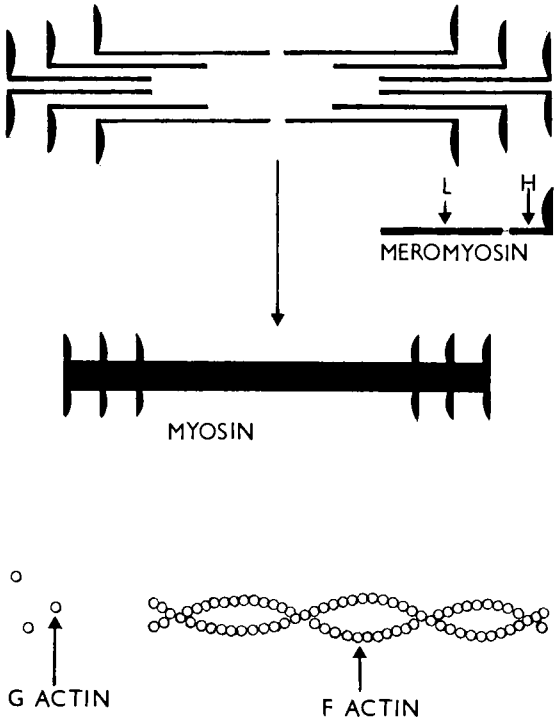

FIG. 10.10. A diagram of the molecular organization of the actin and myosin molecules into myofilaments as deduced from high resolution electron microscopy.

contraction of the muscle the process of change of state continues in a cyclical fashion. During the absence of calcium ions (in the relaxed state) the heavy meromyosin remains held in the extended form. These repeated changes in the protein configuration would have the effect of dragging the actin filaments into the A band towards the M band. Full details of this theory, which is only one of several, are given in the book by Bendall listed at the end of the chapter. When the interdigitation of the thick and the thin filaments takes place, there is an overall shortening of the muscle fibre, which is apparent at the optical microscope level as a shortening of the I band region.

If the force of the contraction is to be transmitted from one sarcomere to another along the length of the fibril, then either the actin filaments must run continuously through the Z band or they must be attached to the material of the Z band itself. Recent E/M studies by Knappeis and Carlsen have shown that the Z band material forms a network and that each actin filament is attached to four cross members of this network and that these run into four actin

filaments on the other side of the Z band, in the adjacent sarcomere (Fig. 10.11). Huxley has suggested that the network structure of the Z band may be formed from the protein tropomyosin.

With the most recent high resolution E/M studies of the A band that have been carried out, cross connections in the centre of the myosin molecules have been recognized. These form the region of high electron opacity which has been called the M band. The detailed structure of the M band region, as deduced by Knappeis and Carlsen, is illustrated in Fig. 10.11. It is thought to help maintain the parallel alignment of the myosin filaments and to help guide the actin filaments as they slide in between the myosin molecules.

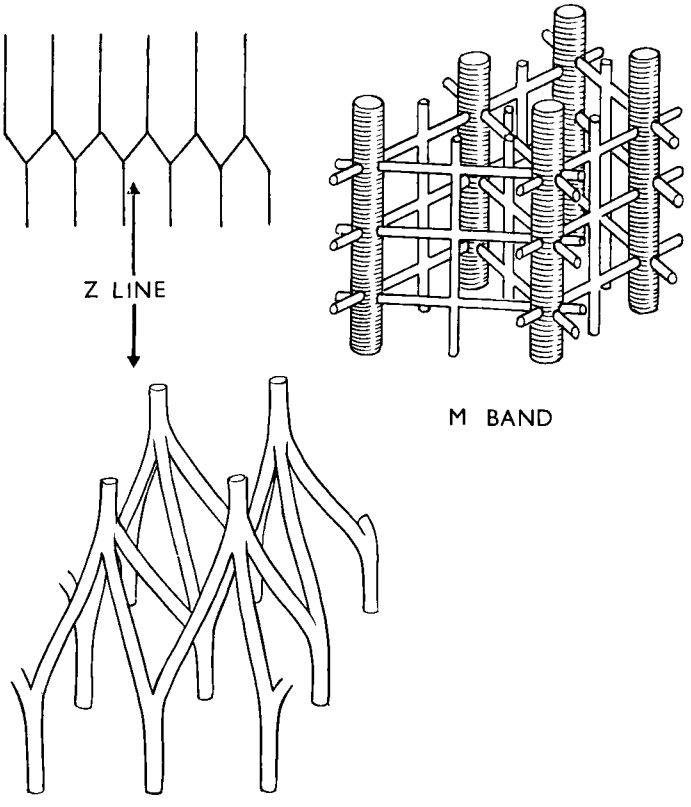

FIG. 10.11. A diagram of the postulated arrangement of the Z band (on the left) and the M band upper right.

Each actin filament is attached to four units of the tropomyosin network of the Z band. The M band region contains an extensive three-dimensional lattice of short M-band filaments attached to each other by transverse M-band bridges. The five thick filaments are myosin molecules which may be held in alignment by the M band structures.

In between the myofibrils the *sarcoplasmic reticulum* is found; this is a branching system of membrane-bound tubules which extends from each Z band to the next. At the level of the Z band there is a swollen terminal cisterna of the sarcoplasmic reticulum which comes into close contact with infolded tubules of the sarcolemma. These latter are the T-tubules which, together with the terminal cisternae of the sarcoplasmic reticulum of two adjacent sarcomeres, forms the so-called *triad* (Fig. 10.12). In the frog skeletal muscle, the T-tubules are at the level of the Z bands, whereas in mammalian skeletal muscle there are two T-tubules per sarcomere, one at each A/I band junction. The T-tubules are now thought to play a

vital role in conducting the excitation inwards from the sarcolemma, this then causes the sarcoplasmic reticulum in some manner to release calcium ions which trigger off the contraction of the myofilaments. The calcium ions are thought to be recaptured by the sarcoplasmic reticulum on the cessation of contraction and passed to the outside by an ATP-powered "calcium pump".

Skeletal or striated muscle is characterized by the presence of numerous mitochondria arranged in rows in the sarcoplasm of each sarcomere between the myofibrils. They also are found in the sarcoplasm just beneath the sarcolemma and in the area of sarcoplasm at the pole of each nucleus. In striated muscle, there are numerous nuclei which are arranged at the

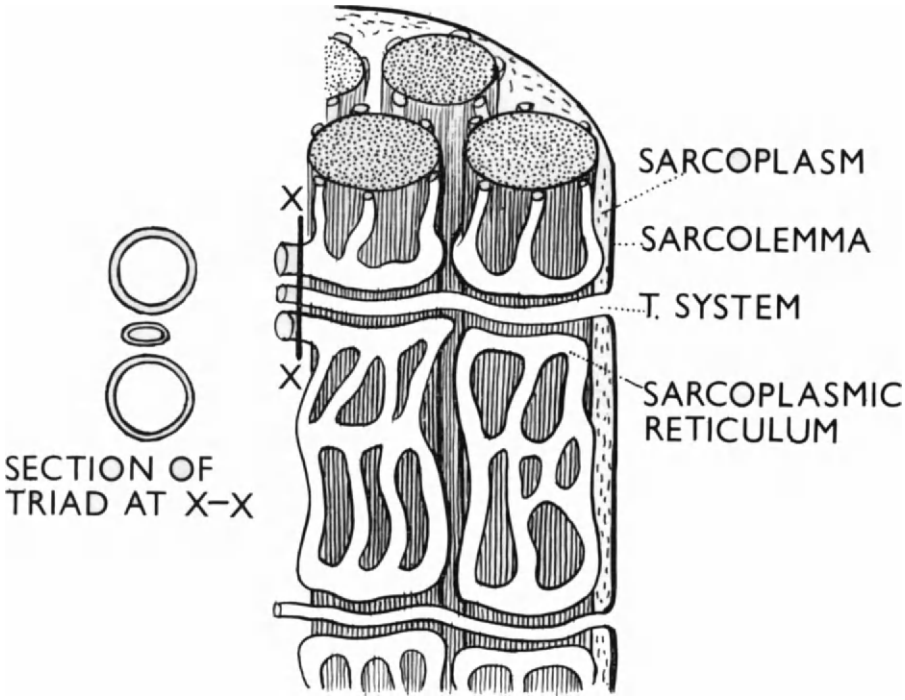

FIG. 10.12. A diagram of the arrangement of the sarcoplasmic reticulum in striated muscle. A section of a triad is shown at X—X; notice the expanded blind ends of sarcoplasmic reticulum vesicles are larger than the T-system invagination. Compare this with the arrangement in cardiac muscle (Fig. 10.18).

periphery of the fibre, immediately beneath the sarcolemma. This may be seen especially clearly when transverse sections of striated muscle are examined (Fig. 10.6) and is a valuable point to bear in mind when trying to distinguish striated skeletal muscle from cardiac muscle under the microscope.

Blood Vessels and Lymphatics

The large arteries and veins are found in the perimysium. The blood supply to muscle fibres is extremely rich, and networks of anastomosing capillaries enclose the fibres in meshes elongated in the direction of the length of the fibres (see Fig. 10.13). The branches of the veins, even the smallest, possess valves. The detailed intra-muscular vascular pattern varies considerably from muscle to muscle.

Lymphatics accompany the blood vessels of the epimysium and perimysium, but are not found between the muscle fibres.

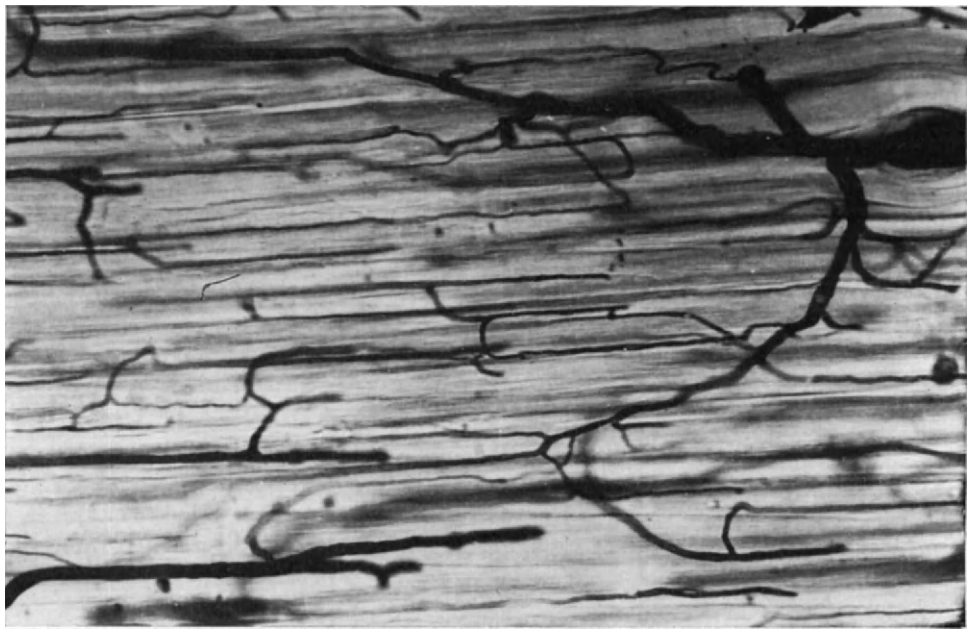

FIG. 10.13. A photomicrograph of a thick section of striated muscle in which the capillaries have been injected. Notice the abundance of anastomoses, a feature of difference from cardiac muscle.

30 μm.

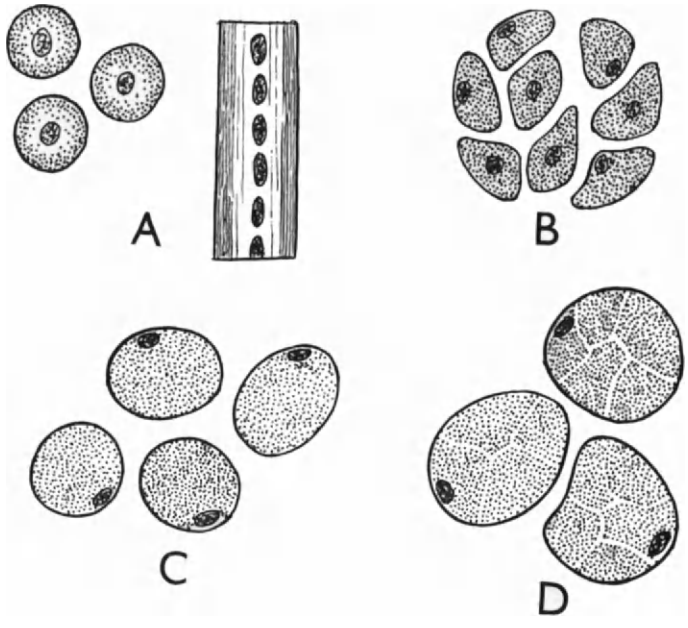

FIG. 10.14. Diagrams of the stages of embryogenesis of striated muscle.

A, Early stage with long chains of nuclei and the contractile filaments at the periphery of the fibres.
B, Later stage; the contractile filaments occupy the whole of the fibre.
C, Nuclei becoming subsarcolemmal.
D, Fully mature fibres with suggestion of division into Cohnheim's areas.

Nerves. Striated muscle is supplied by myelinated nerve fibres. The motor and sensory nerve endings are described later (see pp. 157, 160).

Histogenesis (Fig. 10.14)

Striated muscle tissue is developed from mesoderm in the embryo. The cells elongate, become spindle-shaped, and arranged in parallel bundles, the cells at first multiplying by mitosis to form *myoblasts*. These become converted into the multinucleate muscle fibres by the fusion of several myoblasts to form a syncitium. The myofibrils appear in rows parallel to the long axis: in the periphery of the cell they are already forming by the eighth foetal week. At this stage the muscle elements consist therefore of long cylindrical cells, with many centrally placed nuclei surrounded by undifferentiated cytoplasm, while the periphery of the cells is occupied by fibrils. The fibrils increase by longitudinal splitting, and finally fill the cell so that the sarcoplasm is very greatly reduced in amount: by the twenty-second foetal week the nuclei are being gradually pushed to the periphery. Later still the sarcolemma is differentiated from the surrounding connective tissue.

The muscular tissue increases during foetal growth by an increase in length and girth of the original fibres, by a further differentiation of more myoblasts, and lastly by a longitudinal splitting of the original fibres. After birth, increase in muscle size is due only to enlargement of the fibres already present.

Reaction to Various Conditions

(*a*) **Hypertrophy.** Great activity of a muscle is attended by an increase in its volume, due to an increase in the amount of sarcoplasm present in the existing fibres: in this way the individual fibres are increased in size: there is no formation of new fibres.

(*b*) **Repair of Damaged Muscle.** Injured muscle fibres can repair the part of the cell damaged, provided that the sarcolemmal tubes are still intact, but new muscle fibres are probably not formed to replace any that are *totally* destroyed. Regenerating muscle fibres arise as continuous outgrowths from the stumps of old damaged fibres. Any degenerated remains are removed by macrophages, and invading fibroblasts construct a new endomysial tube into which the regenerating muscle fibre grows. In the absence of sarcolemmal tubes any considerable defect is made good by a connective tissue scar.

The normal health of a muscle depends on the integrity of its nerve supply: a denervated muscle undergoes rapid atrophy, characterized by fatty degeneration of the muscle fibres and increase of interstitial connective tissue. In the same way the nervous connection is necessary for repair of damaged muscle tissue.

Chief Distribution of Striated Muscle

Muscles attached to the bony skeleton and to certain viscera.
Upper third of the external muscle layer of the oesophagus.
Diaphragm.
Muscles in the substance of the tongue.
Extra-ocular muscles.
Muscles of facial expression.

(3) CARDIAC MUSCLE

Cardiac muscle consists of peculiar striated muscle fibres with fibrils running longitudinally as in voluntary striated muscle. It differs from the latter in that there are usually only one or two nuclei per fibre, the nuclei are placed centrally instead of peripherally, the transverse striations are not so definite, and the fibres branch and anastomose (Figs. 10.15, 10.16). Cardiac muscle fibres are usually between 50–100 μm. in length and about 20 μm. in breadth.

The mitochondria are larger and more numerous than in skeletal muscle and they have, in general, larger cristae. There are also differences in the morphology of the sarcoplasmic reticulum and the T-system as compared with skeletal muscle (Fig. 10.18).

The sarcoplasm of cardiac muscle fibres is abundant. The region surrounding the nucleus contains no fibrils, and here the sarcoplasm accumulates in a mass elongated in the direction of the long axis of the fibre: the nucleus (or nuclei, for often the fibres are binucleate) is situated in the middle of this mass. The fibrils are most closely packed round the periphery of the fibre

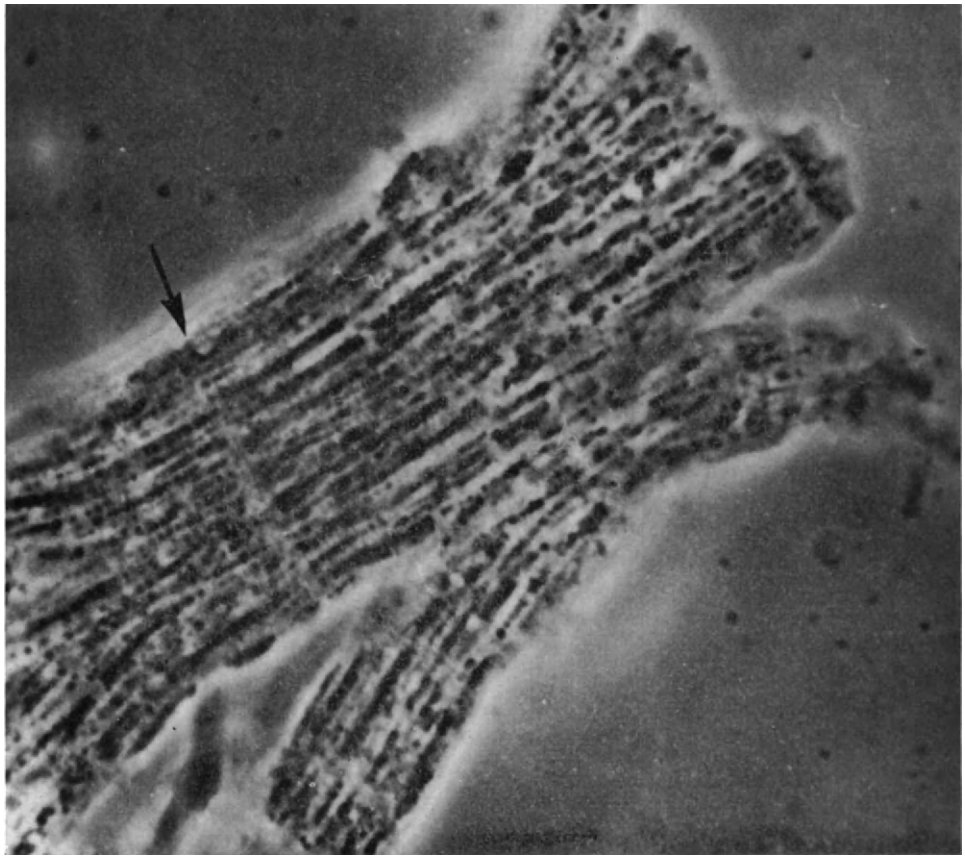

FIG. 10.15. Isolated cardiac muscle cells seen by phase contrast microscopy. The branching of the fibres is apparent, as are the numerous mitochondria (sarcosomes) which appear as dark granules. The arrow indicates an intercalated disc at the boundary between two cells.

and are arranged in groups giving an appearance in cross section rather like that of the Cohnheim's areas in skeletal muscle. The fibres show transverse markings, the *intercalated discs* (Figs. 10.15, 10.17), which occur at the I band level. They appear in living material, as seen under the phase contrast microscope, and in fixed material stained with iron haematoxylin or impregnated with silver nitrate. One disc does not always extend straight across the fibre but may pursue a stepped course. The E/M has shown that the intercalated discs, which are usually at the level of the Z band, represent the cell membrane of the cardiac muscle cell, with its associated membrane areas of specialization. The cell membranes themselves cannot of course be seen with the optical microscope but some material associated along the cytoplasmic

side of the membrane stains very densely and renders the complex visible. This material is known as the *myofibrillar insertion plaque* as it receives the termination of the actin filaments. In addition to this plaque, tight junctions and desmosome-like structures are also visible (Fig. 10.19). One or other of these is almost certainly the site of the electrotonic transmission of the impulse which occurs between adjacent cardiac muscle fibres.

Cardiac muscle cells possess a very large and prominent T-system of tubules extending inwards towards the centre of the muscle fibre from the sarcolemma. They are usually found at the level of the Z bands. The T-system is much more prominent than in skeletal muscle and it is lined with a continuation of the basal lamina which surrounds the sarcolemma. Con-

FIG. 10.16. The typical appearance of cardiac muscle in section. The preparation has been stained with iron haematoxylin and the intercalated discs appear as very dark lines crossing the fibres. The darkly stained circular profiles at the bottom of the picture are some of the red blood cells in a capillary. 25 μm.

versely, the sarcoplasmic reticulum appears to be less complex in cardiac muscle than in skeletal, the large terminal cisternae often being absent. Only small areas of contact exist between the sarcoplasmic reticulum and the T-system (Fig. 10.18) and in E/M sections there is often the appearance of a *dyad* rather than a triad.

Between the fibres is found connective tissue, with its collagen and elastic fibres, and the usual connective tissue cells, together with a very rich blood capillary network.

In addition to the typical myocardial fibres, there is a system of somewhat atypical fibres that constitute the tissue of the heart specially concerned with conducting the impulse for contraction from one part to another: these are the so-called Purkinje fibres, described under "Heart" (see p. 195).

Blood Vessels and Lymphatics

The blood vessels are derived from the coronary arteries and provide a very profuse basket-like capillary network surrounding the muscle fibres. There are numerous anastomoses between

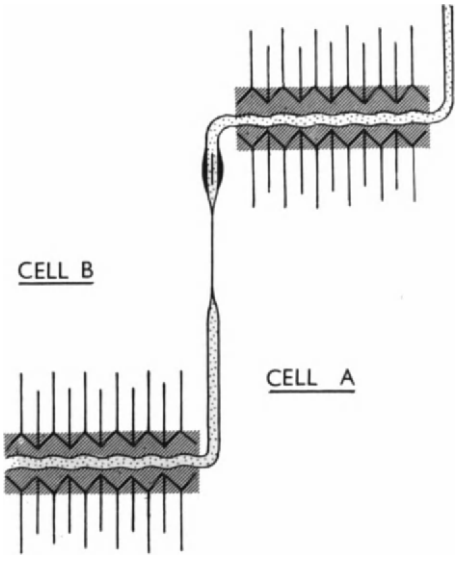

FIG. 10.17. A diagram of the junctional region (intercalated disc) between two cardiac muscle cells. The dense stipple represents the myofibrillar insertion plaque, whilst in the vertical region between the cells a tight junction and a desmosome are indicated.

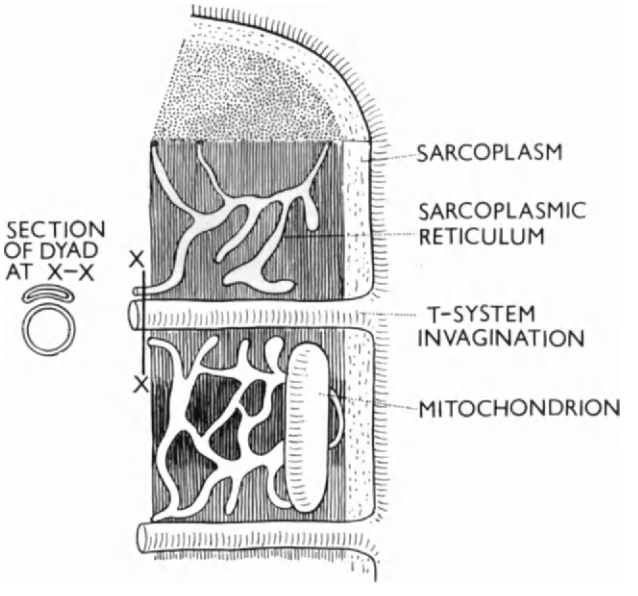

FIG. 10.18. A diagram of the arrangement of the sarcoplasmic reticulum and the T-system in cardiac muscle. A dyad is sectioned at X—X; note that here the T-system invagination is larger than the sarcoplasmic reticulum vesicle.

arterioles of the cardiac muscle but these are, however, not adequate to provide adequate vascular supplies in the event of a sudden occlusion of one of the vessels. The interstitial connective tissue contains a rich supply of lymphatic vessels.

Nerves

The cardiac muscle is richly supplied with non-myelinated and myelinated autonomic nerve fibres: actual endings are often hard to make out, but the efferent fibres make a fine network with varicose swellings and end in enlargements on the surface of the muscle fibres.

Histogenesis of Cardiac Muscle

Heart muscle is developed from the splanchnic mesoderm which immediately surrounds the thin-walled tube of endothelium that at first represents the heart. The myofibrils arrange themselves in groups to form fibres, the nuclei remaining in a central core of sarcoplasm. The intercalated discs appear later, just before the end of foetal life.

FIG. 10.19. An electron micrograph of the junction between two cardiac muscle cells. The electron-dense myofibrillar insertion plaque may be seen at the left. Some of the mitochondria with very prominent cristae which characterize heart muscle are visible on the right. 0·75 μm.

Reaction of Cardiac Muscle to Various Conditions

(1) **Hypertrophy.** In response to continuously increased functional demand, cardiac muscle fibres can enlarge both in length and in thickness: any proliferation of fibres by longitudinal splitting probably plays only a small part in the increase of bulk.

(2) **Atrophy and Damage.** Actual trauma of cardiac muscle is repaired by ordinary connective tissues forming a scar, the muscle having very little power to regenerate. Atrophy of the fibres occurs normally in old age and in starvation, the fibres becoming more slender owing to the disappearance of some of the fibrils.

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CHAPTER 11

NERVOUS TISSUE

The nervous system of the body, both central (brain and spinal cord) and peripheral (ganglia and peripheral nerves), consists of the following tissues:

(1) True nervous tissue, comprising the neurons and their processes, the axons and dendrites.

(2) Interstitial tissue of the nervous system, including all the glial elements.

(3) Connective tissue proper, including the meninges and the coverings of the blood vessels associated with the nervous system.

The relation of these structures to one another is understood most readily by a consideration of their development; a very short account of their histogenesis is therefore given here.

Histogenesis of Nervous Tissues

The neural tube, lined by a simple epithelium, is pinched off from the ectoderm on the dorsal surface of the embryo. At the same time other cells break off from the ectoderm to form two longitudinal bands, the neural crests, lying dorsilateral to the tube between it and the ectoderm which has reunited in the mid-dorsal line so as to enclose the neural tube within the embryonic body. Later, the epithelium of the neural tube differentiates to give rise to the ependyma, the neuroglia and the neurons of the central nervous system, while the neural crest cells differentiate into the elements of the cranial and spinal ganglia: the sympathetic ganglia are derived partly from neural crest cells and partly from cells migrating out from the central grey matter. The surrounding mesenchyme gives rise to the meninges and to the connective tissue of the central nervous system, of the peripheral nerves and ganglia, and also to the microglia.

The columnar epithelial cells of the neural tube very early differentiate into two types of cells, (A) columnar cells or *primitive spongioblasts*, and (B) large round *germinal cells*. The columnar cells multiply, becoming elongated: some of the cells persist as the ciliated lining of the cerebrospinal canal, the *ependyma*, whilst others send processes to the surface which end in small expansions at the external limiting membrane. These latter cells are the *spongioblasts* which will give rise to *neuroglia*; they lose their attachment to the internal limiting membrane: some retain their surface contact and are transformed into the sub-pial neuroglia (p. 172), while others lose this attachment also and become differentiated into free neuroglia (p. 167). The germinal cells multiply rapidly; most of them give rise to *neuroblasts*, while others produce more *spongioblasts*. Later, the neuroblasts develop into neurons, and the spongioblasts into astroglia and oligodendroglia.

To sum up, neuro-epithelium differentiates into two types of cell

(A)	(B)
Columnar	Germinal
↓	↓
(1) Ependyma	(1) Neuroblasts → Neurons
(2) Spongioblasts → astroblasts and oligodendroblasts	(2) Spongioblasts → astroblasts and oligodendroblasts
↓	↓
astrocytes and oligodendrocytes	astrocytes and oligodendrocytes.

This is the only source of the neurons of the central nervous system: those of the dorsal root (cerebrospinal) ganglia are derived from similar cells in the neural crest group. The neuroblast cells (which are easily distinguished from the other elements as they stain more deeply, particularly by silver methods) very soon elongate, each putting out one long process, the axon. The neurofibrils develop, and later first one and then usually several more processes grow out and the Nissl bodies appear.

For details of the growth of the nerve fibres, and further differentiation of nerve cells and nerve cell groups, reference should be made to text-books of embryology.

The primary function of nervous tissue is to receive stimuli from the outside, to transform them into nervous impulses, and to convey these to other parts of the body so that a suitable response may occur: hence the development of this tissue from the ectoderm. In highly developed organisms such as the human the nervous system has become very complex, and its centres of activity are concentrated for the most part in the well-protected brain and spinal cord, but these centres are still in connection with the outer surface and with those organs that carry out the responses. It is for this reason that nervous elements are in many cases very elongated.

The unit of morphological and functional structure is the *neuron*, which may be defined as a nerve cell with all its processes. The processes, which are parts of the cell, are often very long; those conducting impulses into their cell of origin being called *dendrites*, and the one conducting the impulse away from its cell being the *axon*. The structure of all the processes is fundamentally the same; the difference between them is a functional one. A nerve path consists of a chain of neurons, varying from two up to a very large number; connection between adjacent neurons is effected by a *synapse*, the conduction of a nerve impulse across a synapse occurring in one direction only. The axon of one neuron ends by branching and providing numerous minute end-bulbs which are distributed over the surface of the cell body and proximal portion of the dendrites of the next neuron (the *boutons terminaux*) (Fig. 11.15); these junctions constitute the synapses. Nearly half the cell surface can be used to provide the synaptic area, the intervening area being used for the interchange of metabolites.

The usually accepted "*neuron theory*" maintains that every mature nerve cell is a discrete unit, the cell consisting of the cell body with nucleus and all the processes. There is no actual continuity of substance between neurons. If a neuron is injured, the direct effects are confined to that particular neuron, and if any part is cut off from the cell body that part will die. For a discussion of the mechanism of nervous conduction and of the "neuron theory" reference should be made to physiology text-books.

A. THE NEURON

The nerve cell consists of a nucleated cell body (the *perikaryon*) which is drawn out into the long processes which form its most characteristic feature. Neurons occur within the grey matter of the brain and spinal cord, and in the ganglia of the cranial and spinal nerves and of the autonomic system.

The cells vary in size from the small granule-cells of the cerebellum, with a diameter of about 5 μm., to the large motor cells of the anterior horn of spinal grey matter that may reach 130 μm. in diameter. The shape of the cell may be spherical, pear-like or multangular, depending on the number and point of origin of the processes, and on the influence of surrounding structures. There is at least one process, the *axon*, which becomes the axis cylinder of a myelinated or unmyelinated nerve fibre: this process carries the nerve impulse away from the cell. There are usually other processes which carry nerve impulses into the cell: these frequently branch quite close to the cell body and are consequently known as *dendrites*.

Morphological Classification of Nerve Cells

Neurons are usually classified according to the number of their processes into unipolar, pseudo-unipolar, bipolar and multipolar cells (Fig. 11.1).

(*a*) **Unipolar Cells.** All developing neuroblasts pass through a stage when they have only one process, the axon. In the adult human such true unipolar cells are very uncommon, but they are found in the mesencephalic nucleus of the fifth cranial nerve.

(*b*) **Bipolar Cells.** Typically these cells are spindle-shaped, having the axon at one pole and a dendrite at the other. Many of the developing neuroblasts pass through this stage, and in the adult they are found in the retina, in the spiral ganglion of the cochlea, in the vestibular ganglion, and in the olfactory neuro-epithelium.

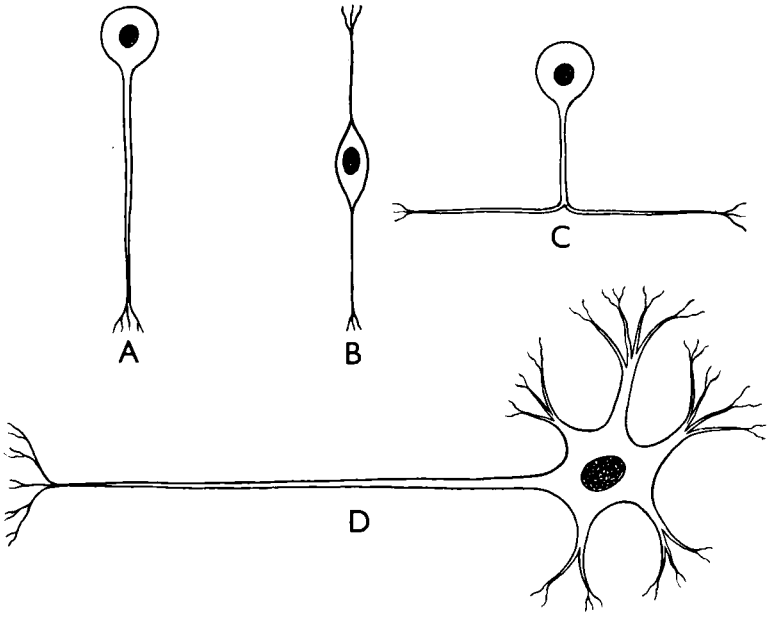


FIG. 11.1. A diagram to show the basic morphological features of the types of neuron: A unipolar, B bipolar, C pseudo-unipolar, D multipolar.

(*c*) **Pseudo-unipolar Cells.** These are atypical bipolar cells which are found in all spinal ganglia and in the ganglia of the cranial nerves other than those of the eighth nerve. These cells are at first typically bipolar and spindle-shaped (Fig. 11.2), but as development proceeds the processes converge until they meet at one side of the cell body: this then elongates so that a fine process is formed with a T-shaped division at the end, one branch of the T being the dendrite from the periphery while the other is the axon extending centrally.

(*d*) **Multipolar Cells.** These cells are of most varied form: a few of the common types are shown in Fig. 11.3. In general, the shape depends chiefly on the number and position of the dendrites.

A second classification based on the length of the axon separates the neurons into two groups, termed Golgi type I and type II cells respectively. Golgi type I cells have an axon which has an extensive course outside that part of the gray matter in which its cell body lies; these cells constitute the bulk of the neurons which form the peripheral nerves and the main fibre tracts in the CNS. Type II cells on the other hand are those with a short axon which does not leave that part of the gray matter in which its cell body lies and they are to be found in the retina, the cerebellar and cerebral cortices.

A third system classifies neurons, according to their function, into *sensory*, *motor* and *intercalated* (or interneurons). Sensory cells include those neurons which are responsible for receiving stimuli and passing them to the central nervous system; cells in the spinal or dorsal root ganglia are good examples. Motor neurons are directly responsible for the effective control of the muscular system and are found in such areas as the motor area of the cerebral cortex, in the gray matter of the anterior horn of the spinal cord and as Purkinje cells in the cerebellum. Any neurons which are interposed in a neural pathway between sensory and motor neurons would, in this scheme of classification, be called intercalated neurons.

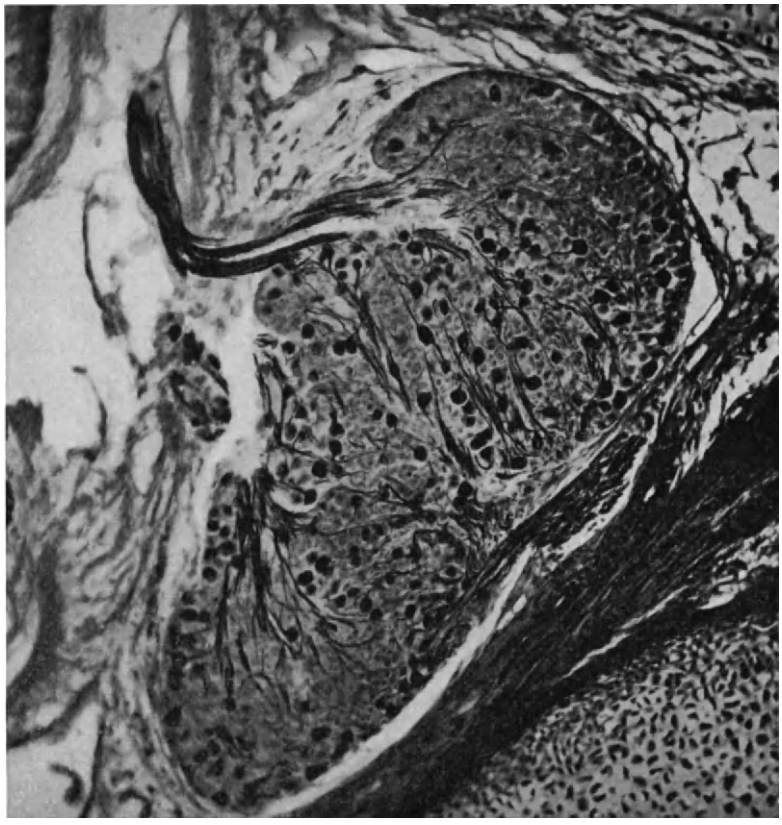


FIG. 11.2. Developing nerve cells in a spinal ganglion of a 10 week human foetus. Silver impregnation. The majority of the cells are still bipolar at this stage but will soon become pseudo-unipolar. 50 μm.

Structure of the Nerve Cell

(*a*) **Nucleus.** The nucleus is usually large, spherical and central, and varies in size with the size of the cell and with its state of activity. The nerve cell nucleus is a particularly suitable one in which to see the sex chromatin of the female which appears as a small dark staining body close to the nucleolus or nuclear membrane. Although present in males, it is too small to be seen under normal conditions.

The particles of chromatin in the nucleus are finely dispersed and hence the nuclei of neurons appear rather pale and "empty-looking" when stained with basic dyes such as thionin. One or two nucleoli are characteristically present.

(*b*) **Cytoplasm.** The cytoplasm of neurons contains the following organelles.

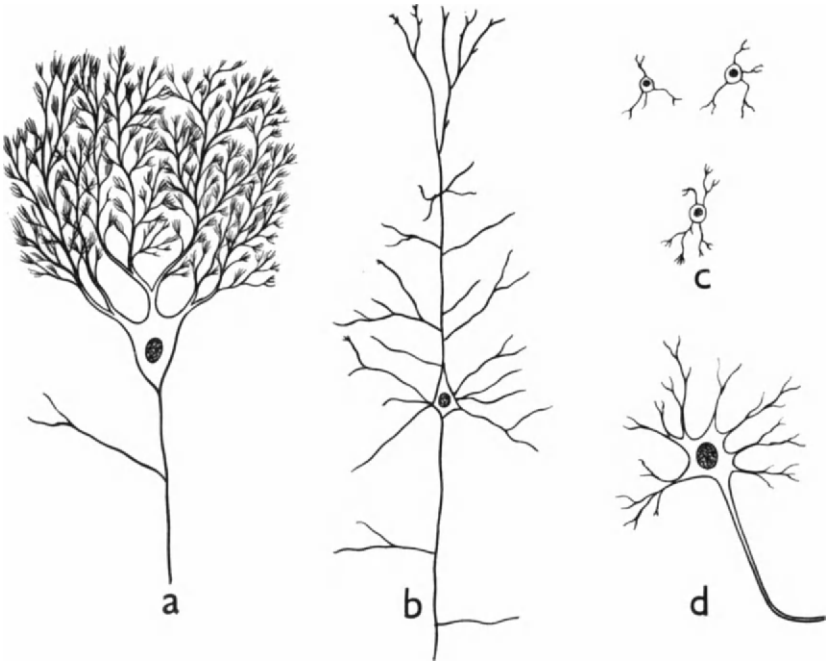


FIG. 11.3. A diagram of some of the common forms of multipolar neuron. a, Purkinje cell of the cerebellar cortex b, pyramidal cell of the motor cortex c, small neuron from the spinal nucleus of the trigeminal nerve d, motor neuron from the ventral horn of the spinal cord.

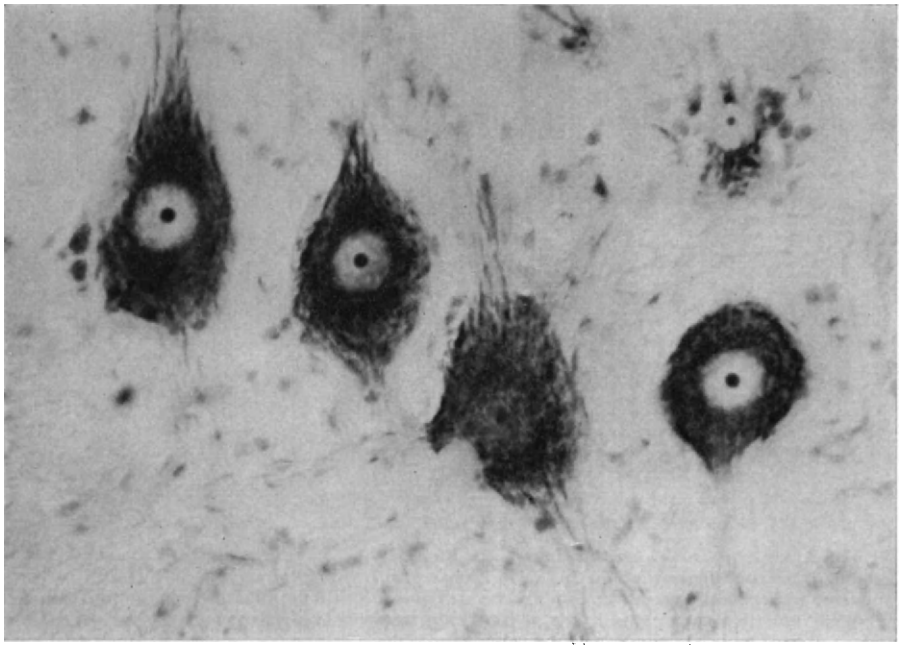


FIG. 11.4. Neurons from the ventral horn of the spinal cord stained with a basic dye. The Nissl bodies may be seen as darkly-staining clumps in the cytoplasm; the nucleolus also stains well with this technique.

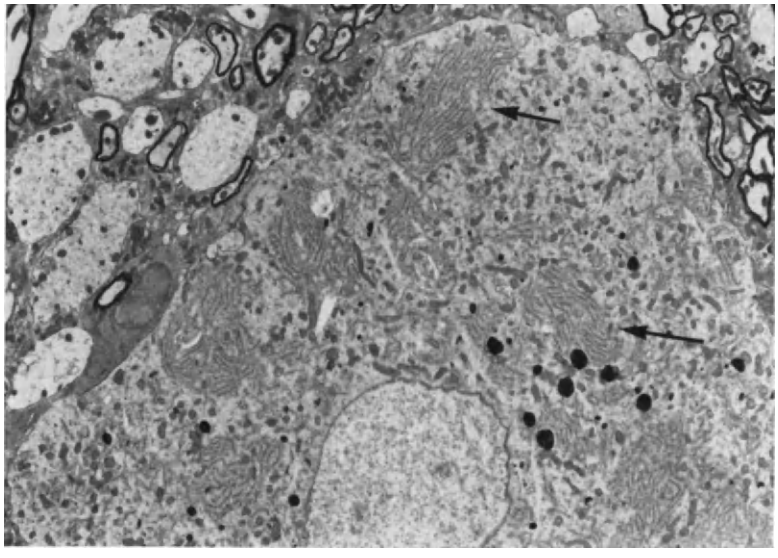


FIG. 11.5A. A low power electron micrograph of a neuron in the central nervous system. The arrows indicate two of the clumps of endoplasmic reticulum which are responsible for the basiphilic staining of the Nissl bodies. 5 μm.

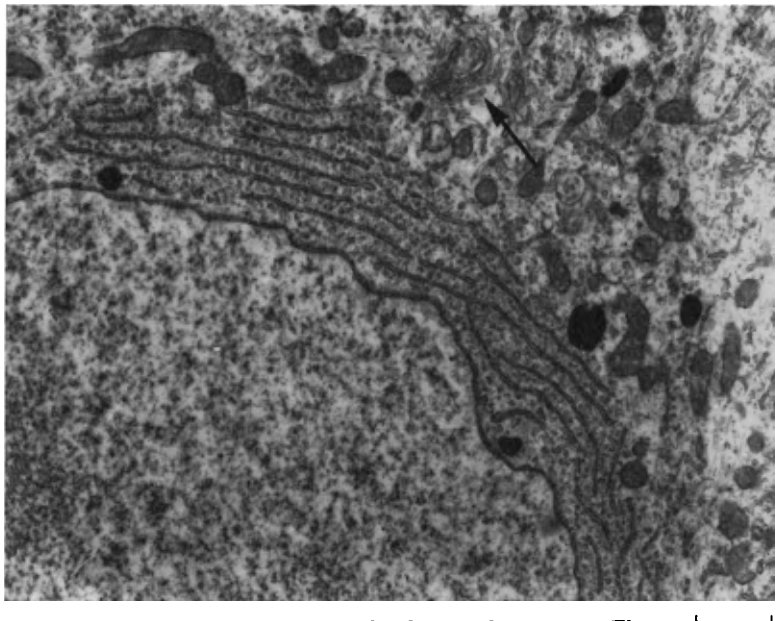


FIG. 11.5B. A high power electron micrograph of part of a neuron. The nucleus is at the bottom left of the micrograph and a clump of rough-surfaced endoplasmic reticulum is close to it. Several mitochondria are visible and the arrow indicates one group of membranes of the Golgi apparatus. Micrographs by Dr. G. Raisman and Pauline Field. 0·5 μm.

(i) *Neurofibrils.* By the application of certain special methods (particularly those involving impregnation with silver salts) all nerve cells can be shown to contain fibrils. They are present in the cell body and extend into all the processes: some threads are fine and some are relatively thick, while the actual thickness may vary with varying activity of the neuron. Within the cell body there is a definite network arrangement of the fibrils. Investigations with the E/M suggest that the neurofibrils of optical microscopy may represent aggregations of very much smaller threads, which have been called "neurofilaments" *c.* 10 nm. in diameter. High resolution electron microscopy has shown that they bear a marked resemblance to microtubules, with walls composed of globular subunits 3 nm. in diameter, arranged in a helical fashion. Their significance is still a matter for speculation.

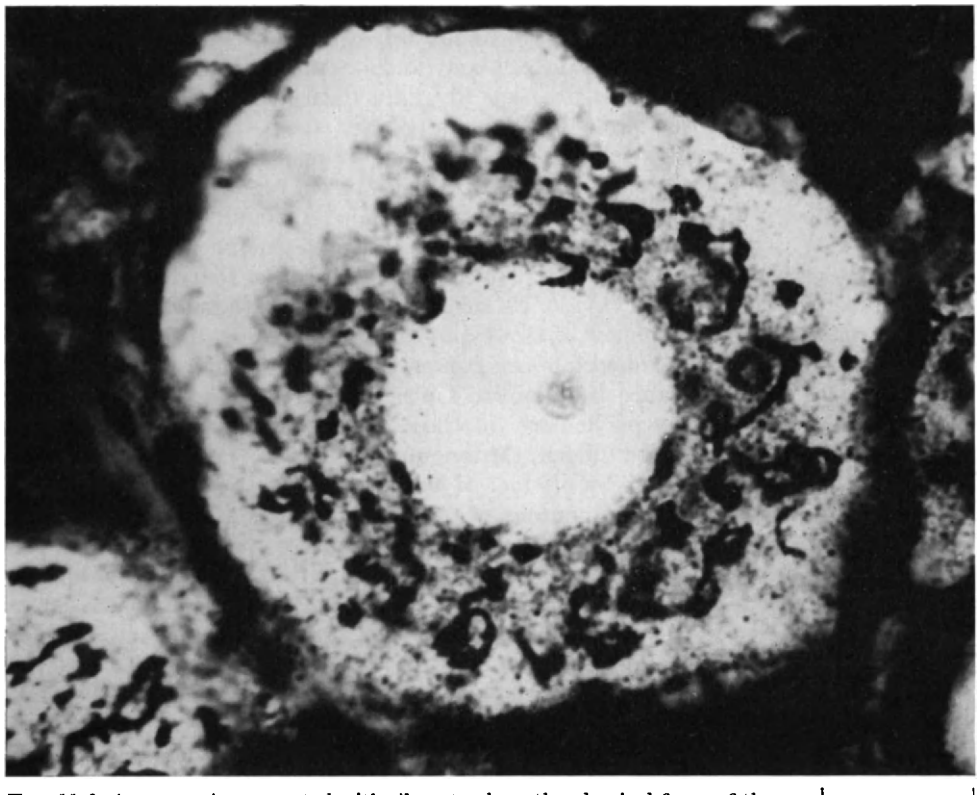


FIG. 11.6. A neuron impregnated with silver to show the classical form of the Golgi apparatus of this cell as a network of anastomosing threads surrounding the nucleus.

(ii) *Nissl bodies.* These are small masses of granular material (Fig. 11.4), found in most neurons, which have an affinity for basic dyes. Nissl bodies are visible in the living neuron by phase contrast microscopy. Histochemical studies have shown that Nissl bodies are rich in ribonucleic acid, whilst E/M studies have shown that they consist of masses of rough-surfaced endoplasmic reticulum (p. 19) highly organized in the form of flattened membranes enclosing cisternae (Fig. 11.5A). The membranes are fenestrated and intercommunicate. Their outer surfaces are studded with ribosomes; it is these which are responsible for the basiphilia noted with the optical microscope.

There is evidence of a high protein turnover, even in the adult functional neuron, and this correlates well with the presence of such an extensive and well organized granular endo-

plasmic reticulum. The Nissl bodies are absent from the layer immediately surrounding the nucleus and from the place of origin of the axon, the *axon hillock*, but they extend into the dendrites. The quantity of Nissl material in the cell is roughly proportional to the size of the cell, but both this and the arrangement of the granules vary in different states of physiological activity. Small nerve cells of the central nervous system sometimes contain no Nissl bodies.

After axon section (see also p. 150) or injury to the perikaryon itself, Nissl bodies change their appearance and may disappear. This phenomenon is known as *chromatolysis*.

(iii) *Mitochondria*. Mitochondria in the form of rods or spherical granules can be seen in all nerve cells when treated by supravital staining, or viewed by dark-ground illumination. They are scattered throughout the cell body and its processes, being especially numerous in the nerve terminals. The mitochondria may be very elongated in axons and cell processes.

(iv) *Golgi Apparatus* (Fig. 11.6). By special methods of fixation and staining the Golgi apparatus can be demonstrated in all nerve cells as a well-developed network of anastomosing threads, varying in its arrangement in the cell body and extending always into the dendrites, but not into the axon. The E/M shows that the Golgi apparatus of the neuron resembles that found in other secretory cells; there are numerous parallel stacks of flattened membranous cisternae surrounded by numerous small vesicles. There are many separate clusters of Golgi membranes arranged around the periphery of the nucleus, linked together by tubular elements which resemble those of the smooth (agranular) endoplasmic reticulum.

(v) *Centriole*. With the optical microscope a typical centrosome and centriole cannot be seen in neurons; with the E/M, however, a typical centriole is often found in the cytoplasm, even though the neuron will never divide. Its role in the neuron is therefore very obscure.

(vi) *Pigment*. Granules of pigment of two types can be distinguished. Melanin is present as dark brown or black granules in certain cell groups, notably in the substantia nigra and in the locus caeruleus: its significance is unknown. Lipochrome granules of a yellow or orange colour are found in some cells, particularly in those of the autonomic ganglia, and more especially in the region of the axon hillock: the amount of this yellow pigment increases with age, suggesting that it represents a by-product of normal metabolic activity.

(vii) *Neurosecretory material*. The neurons of the *supraoptic* and *paraventricular nuclei* of the hypothalamus contain droplets with characteristic staining properties. These are present not only in the cell bodies but also in their axons, which terminate in the neural lobe of the hypophysis (see Chapter 16). There is strong evidence suggesting that the stainable substance is a precursor or carrier of the neurohypophysial hormones. It is by no means improbable that other kinds of neuron possess a secretory function.

(*c*) **Cell Membrane.** The surface or plasma membrane of a neuron appears to be basically similar to that of cells in general (see Chapter 2). Within the central nervous system the cells are closely invested by a network of neuroglial cells and fibres, while in the peripheral ganglia the nerve cells are surrounded by a capsule of satellite cells of similar origin.

(*d*) **Cell Processes.** (i) *Dendrites*. These are the processes which conduct the impulse into the neuron, i.e. they are *afferent*. They show tremendous variation in size and complexity but usually they provide the bulk of the receptive area of the neuron surface. Dendrites may be distinguished microscopically from the efferent process (the axon) by the fact that Nissl bodies and mitochondria are both present in the dendrites. It is common to find the dendrites arborizing or branching many times, in a pattern which is typical for each type of neuron (Fig. 11.3). The E/M shows that most dendrites contain neurotubules.

(ii) *Axons* (*Axis cylinder*). The axon is the efferent process of the neuron; usually there is only one per neuron (whilst there are many dendrites), and the axon is larger and less branched. Nissl bodies are absent from the axon, but neurotubules and mitochondria are present. There is a very variable type of arborization at the axon terminal.

Axons are often distinguished by their ensheathing layer of myelin which represents a proliferation of the cell membrane of the surrounding Schwann cell.

B. NERVE FIBRES

Nerve fibres (the axons together with any associated coverings or sheaths) are of two kinds, myelinated and unmyelinated. The white matter of the central nervous system consists largely of myelinated fibres, as do also the cranial and spinal nerves: most of the autonomic fibres, particularly the post-ganglionic ones, are unmyelinated. In both types of fibre the axon is the essential component.

(1) Myelinated (medullated, white) Nerve Fibres (Fig. 11.7)

These fibres consist typically of a central core (*axon*) surrounded by a sheath (*myelin sheath*), which is itself surrounded, except within the central nervous system, by a delicate

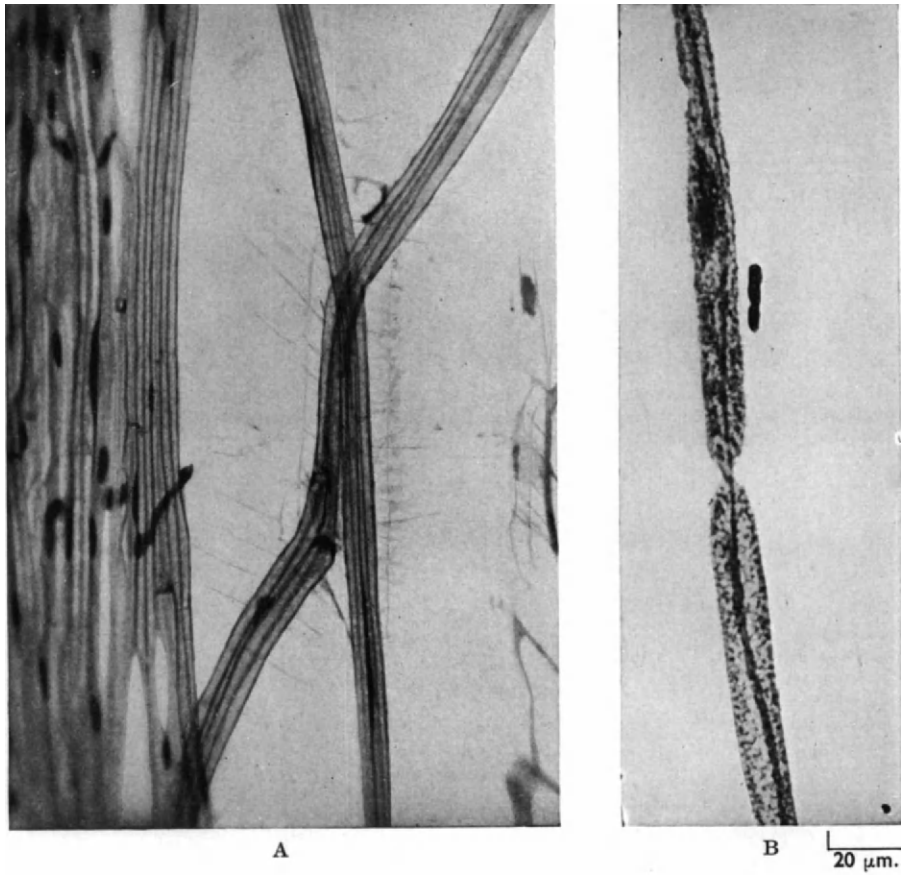


FIG. 11.7. Teased preparations of nerve fibres from a cat. A, stained with Giemsa; the nuclei of the Schwann cells may be clearly seen. B, stained with haematoxylin and eosin to show the axon inside the myelin sheath.

sheath consisting of the cytoplasm of the very much flattened nucleated *Schwann cells*. The myelin sheath is lost near the peripheral termination of the fibre. External to the Schwann cells and closely adhering to them is a very delicate connective tissue membrane, the *neurilemma** derived from the endoneurium of the nerve.

* Some authors regard the layer of Schwann cells as constituting the *neurilemma* and what is here called the neurilemma is referred to by them as the *sheath of Henle*.

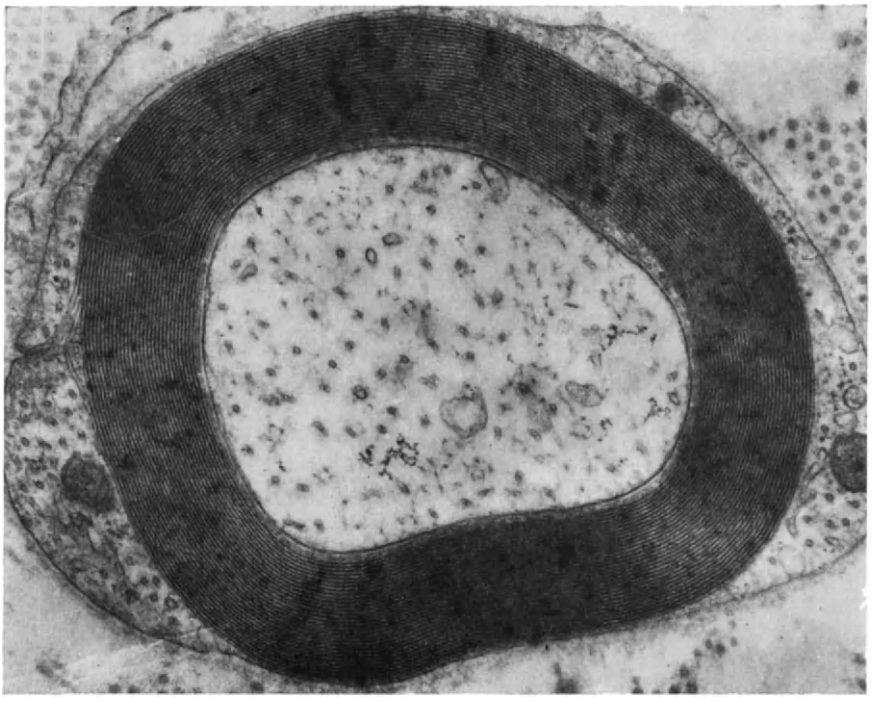


FIG. 11.8A. An electron micrograph of a myelinated nerve fibre in transverse section. The periodicity of the myelin sheath is well shown, as is the investing cytoplasm of the Schwann cell. Micrograph by Dr. J. Morris. 0·5 μm.

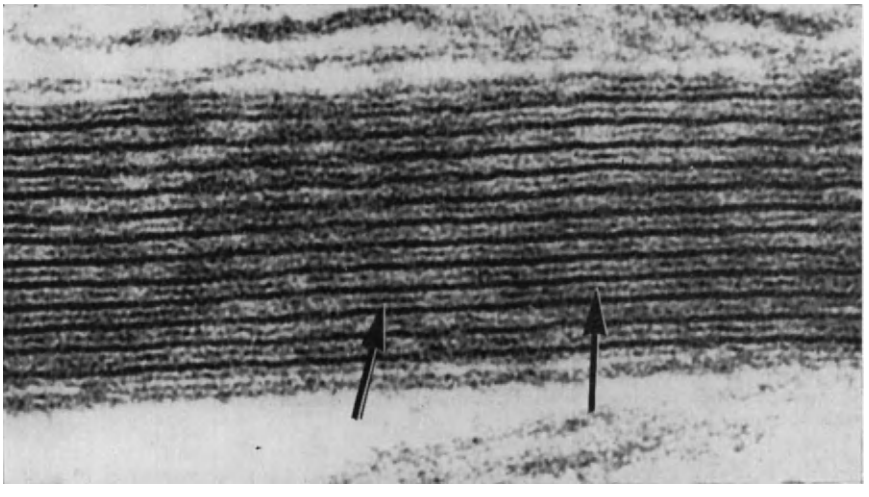


FIG. 11.8B. A high-power micrograph of part of the myelin sheath of a nerve fibre. The thick major dense line is clearly visible and the arrows indicate one of the thin intraperiod lines which represent the union of the outer lamellae of the unit membrane of the Schwann cell. 500 Å

The myelin sheath is composed of lipoprotein; in the fresh condition it appears white and highly refractile. The myelin sheath blackens slowly with osmium tetroxide, showing the presence of some unsaturated lipids, and it is dissolved by the usual lipid solvents. This accounts for the fact that it is difficult to demonstrate the myelin sheath in ordinary paraffin sections.

Studies over the last twenty years by polarized light, biochemical analysis techniques and by X-ray diffraction have suggested the presence of a lamellar structure in the myelin. Recently the E/M has allowed this to be visualized directly (Fig. 11.8). The lamellae appear to be arranged concentrically around the axon. Current opinion holds that each lamella is derived from the plasma membrane of the Schwann cell so that it is not surprising that the molecular arrangement in the myelin lamellae turns out similar to that in the cell membrane itself, i.e. with bimolecular leaflets of phospholipoprotein. A study of the high resolution

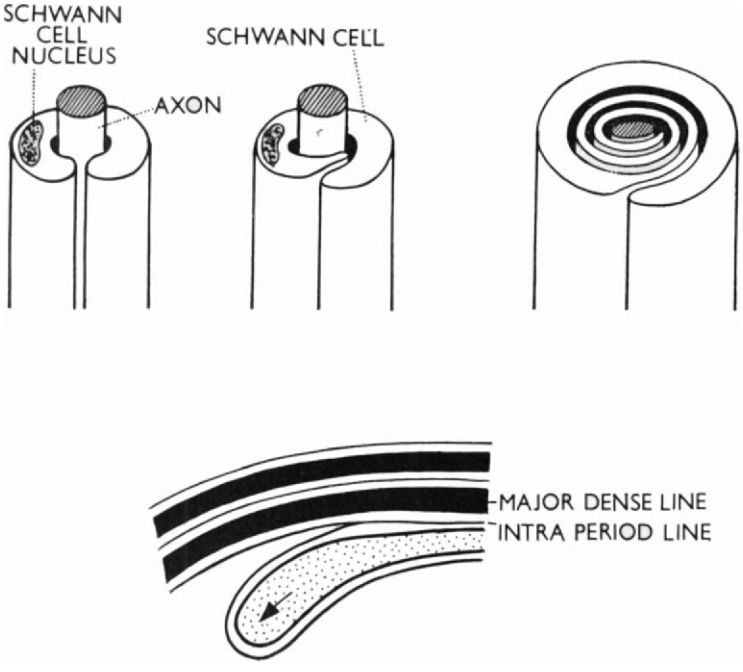
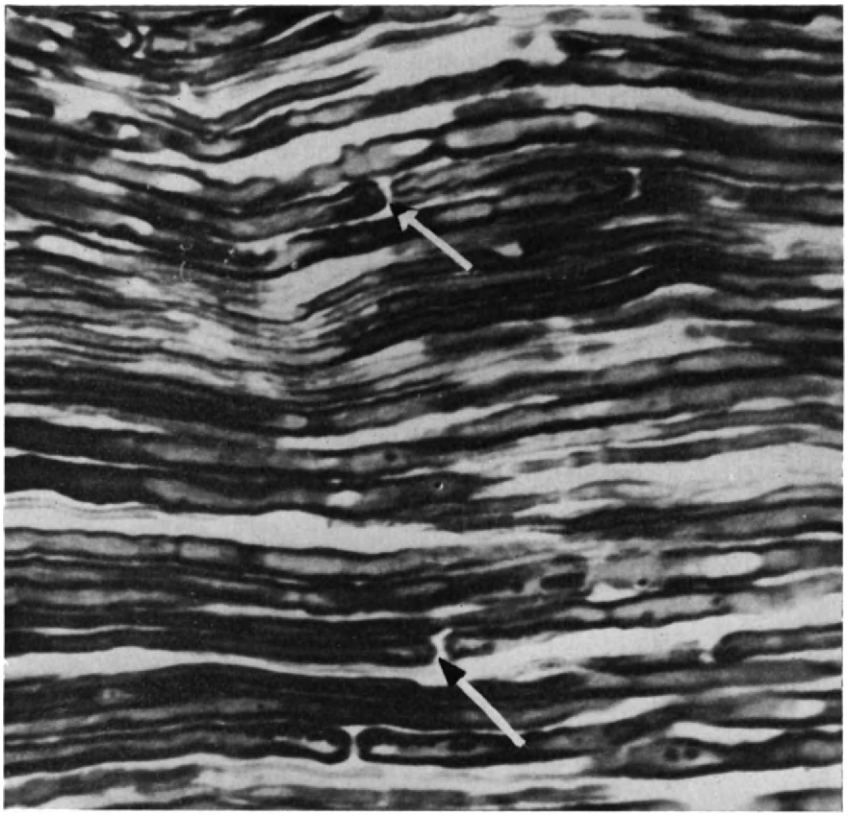


FIG. 11.9. A diagram of the current concept of myelination of an axon. The major dense line and the intraperiod line are formed by the fusion of the cytoplasmic lamellae and the outer lamellae respectively, of the Schwann cell unit membrane, as shown in the lower drawing.

electron micrographs which are now available of myelin shows that the lamellae appear as an alternating series of thick and thin dark lines. These are referred to as the major dense line and the intraperiod lines respectively. The major dense line (about 3 nm. thick) represents the apposition of the inner or cytoplasmic surfaces of the unit membrane of the Schwann cell, whilst the intraperiod line represents the union of the *outer* aspects of the same membrane (Fig. 11.9).

To understand the origin of the lamellation it is necessary to know something of the manner of myelin formation. Tissue culture experiments have shown the developing nerve fibres become associated with special cells—the *Schwann cells* previously mentioned. The Schwann cells envelop successive stretches of the growing axon and each stretch, associated with one such cell, corresponds to an *internode* (i.e. the distance between two successive nodes of Ranvier—see below). The axon then comes to lie at the bottom of an invagination of the



20 μm.

FIG. 11.10. An optical micrograph of teased nerve fibres from the sural nerve of a rat. The myelin has been blackened with osmium tetroxide. The arrows indicate two of the numerous nodes of Ranvier.

FIG. 11.11. A diagram of the organization of a myelinated nerve fibre. 1, axon 2, Schwann cell cytoplasm 3, neurilemma 4, node of Ranvier 5, myelin.

cytoplasm of each successive Schwann cell. The Schwann cells then begin to grow around the axon in such a way that their plasma membranes begin to envelop the axon in a spiral sheath and their cytoplasm (or the bulk of it) along with the nucleus is progressively squeezed out, until at the completion of the process it occupies a superficial position. Thus the myelin envelope or sheath consists of the spiralized membranes of a linear succession of Schwann cells. The lamellation is a consequence of the manner in which the lipid and protein components of the successive whorls coalesce.

In peripheral nerve fibres the myelin sheath is interrupted at regular intervals: these interruptions constitute the *nodes of Ranvier*, which appear as constrictions of the fibre (Fig. 11.10) when seen with the optical microscope.

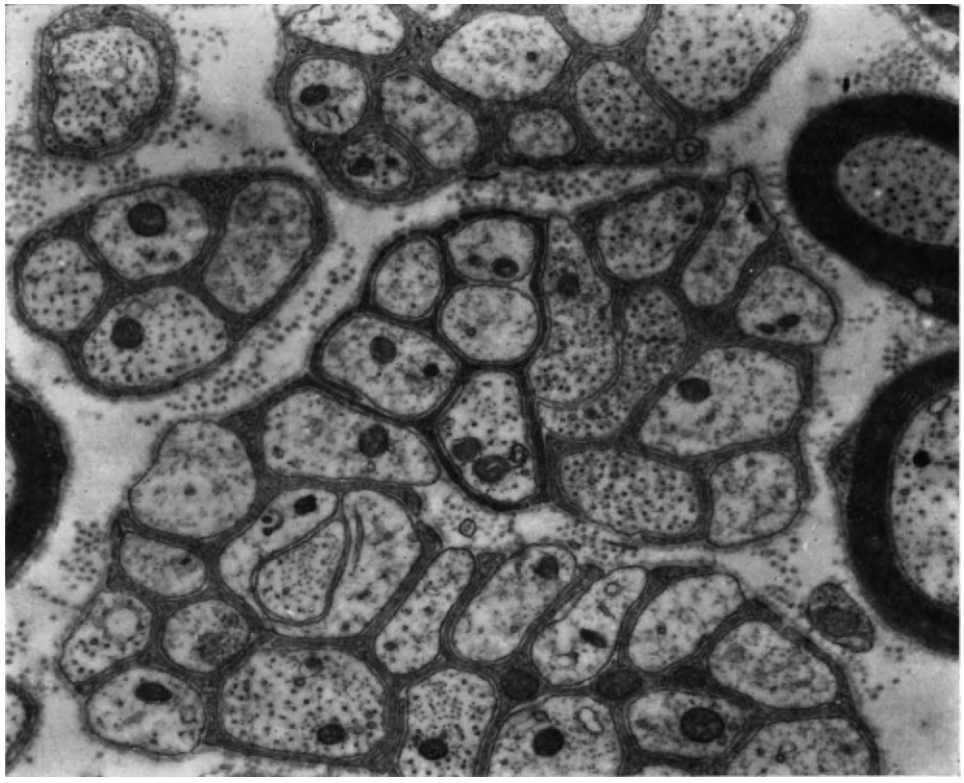


FIG. 11.12. An electron micrograph of a group of unmyelinated nerve fibres in transverse section. A variable number of nerve fibres are enclosed in the cytoplasm of one Schwann cell. The basal lamina can be seen round each Schwann cell. Two myelinated nerve fibres are visible at the right of the micrograph. Micrograph by Dr. J. Morris. 1 μm.

The node of Ranvier is of course the junction between two Schwann cells, where there are only interdigitating processes of Schwann cell cytoplasm and a layer of basal lamina material covering the axon proper. The presence of the myelin sheath greatly increases the fibre conduction speed without requiring any further increase in the axon diameter. This is important as it decreases the reaction time. In the myelinated nerve fibre, resting and action potentials are limited to the nodes of Ranvier and this allows the type of nervous impulse transmission known as *saltatory conduction*. For details of this and other aspects of the physiology of the nervous impulse, a standard textbook of neurophysiology should be consulted.

The cytoplasm of the Schwann cells forms a very thin tube-like membrane covering the

nerve fibre directly overlaying the myelin sheath. It is here that the flattened nuclei of the Schwann cells may be seen (Figs. 11.11, 11.7) causing a small depression in the myelin and a little outwards bulging of the neurilemmal membrane.

The Schwann cells are derived from the neural tube or neural crest; the cell membrane encloses a small amount of protoplasm, a nucleus, mitochondria and a Golgi apparatus. One cell provides the cytoplasmic covering for one internodal segment: thus one nucleus is found between two neighbouring nodes which are the junctions between two Schwann cells.

Within the central nervous system there is no investing sheath of Schwann cells, but filling all the space between adjacent nerve fibres is a dense layer of neuroglia and fibres. Myelination in the central nervous system occurs through the agency of oligodendroglial cell processes, acting in a similar manner to Schwann cells in the peripheral nervous systems.

Myelinated nerve fibres vary very much in size: the largest with a diameter up to 20 μm. supply the skin and striated muscles, while the smallest are the preganglionic autonomic fibres with a cross-section measuring 2 μm. to 4 μm.

(2) Non-myelinated (grey) Nerve Fibres

These fibres are very much smaller and possess no stainable myelin. With the E/M, several of them are usually seen to be enclosed in the cytoplasm of one Schwann cell, and the whole is surrounded by a layer of amorphous material of the basal lamina (Fig. 11.12). It seems probable that the Schwann cells are concerned with the nutrition or some other aspect of the metabolism of the nerve fibre.

Reactions of Nerve Cells to Various Conditions

The difficulty of investigating and interpreting changes in nervous tissues under physiological and pathological conditions is greatly increased by the rapidity with which post-mortem changes set in and by the need for the use of specialized techniques.

(*a*) Physiological Activity

Functional activity of nerve cells has been correlated by various workers with a thinning of the neurofibrils, and with a diminution in the amount of Nissl substance: the Golgi apparatus is not affected. The neurofibrils also become thicker and fewer in extreme cold, thinner and more numerous in heat. If the activity is prolonged to the stage of exhaustion it is said that the Nissl bodies disintegrate, and may even disappear for a time: some workers deny this.

(*b*) Section of the Axon

Injury to the axon always affects the cell of which it forms a part. The neurofibrils and Golgi apparatus may become fragmented and the mitochondria disappear, but the changes that are most readily seen are those occurring in the nucleus and in the Nissl bodies. The latter break down and seem to undergo partial dissolution—*chromatolysis*—so that the cytoplasm stains homogeneously and much less strongly. At the same time the whole cell at first swells up and becomes globular and the nucleus takes up an excentric position: very shortly shrinkage of both cell and nucleus occurs and many lysosomes appear in the cytoplasm. Regeneration of the axon is followed by a return to normal appearance on the part of the cell.

(*c*) Pathological Conditions

Specific changes in nerve cells associated with different diseases are very few. Examples are the accumulation of various glycolipids in neurones of patients suffering from infantile amaurotic idiocy (Tay-Sach's disease) and the chromatolysis, and ultimate necrosis of neurones in the lesions induced by the poliomyelitis virus. Nerve cells cannot multiply by division, and any that are destroyed are not replaced.

Reactions of Nerve Fibres to Various Conditions

(*a*) Section of Nerve Fibres

Changes occur in the cells of origin of injured nerve fibres, and also in the fibres themselves. There is an immediate local degeneration of both central and peripheral parts of

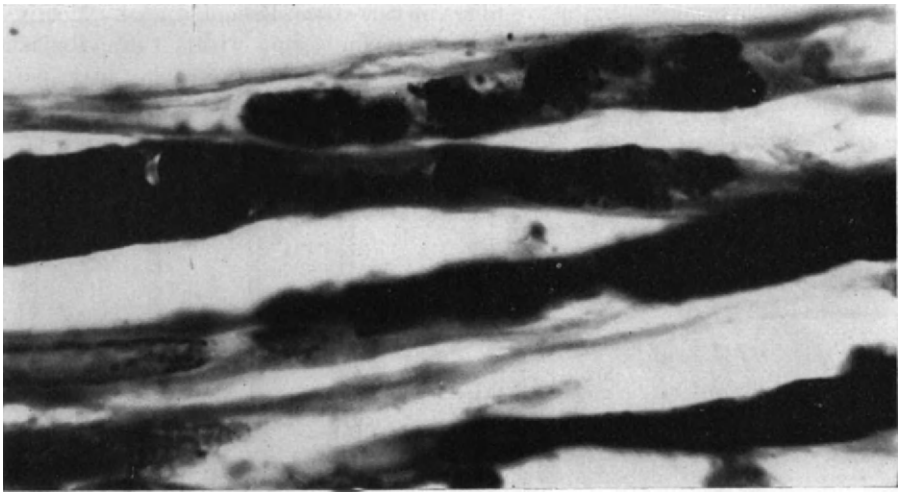


FIG. 11.13A. A micrograph of degenerating nerve fibres. The degenerating myelin stains very darkly; at the left of the picture the endoneurial tubes may be clearly seen.

10 μm.

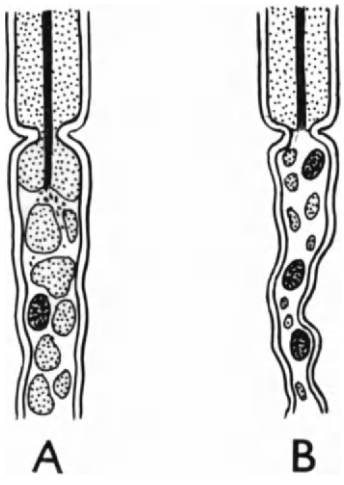


FIG. 11.13B. A diagram of degenerating nerve fibres. A, after two days. B, after a week, when the debris is being removed by the activity of macrophages.

the nerve fibre known as primary or traumatic degeneration. Further, any nerve fibre that is cut off from its cell of origin ultimately degenerates completely, the changes that take place during the process being known as *secondary* or *Wallerian degeneration* (Fig. 11.13). Secondary degeneration affects that portion of the nerve fibre that is actually part of the nerve cell, namely, axon and myelin sheath (if present): the Schwann cell does not depend for its life on the neuron, and it consequently survives to help in the subsequent regenerative processes.

Secondary degeneration affects the whole of the nerve fibre distal to the lesion, and extends back towards the cell at least as far as the next node of Ranvier.

Within one day after the lesion the axon begins to break up, and after about ten days the last granules of it disappear. Within two days after the lesion the myelin sheath fragments into fatty droplets, and chemical breakdown takes place with the formation of substances including highly unsaturated fatty acids: it is these which are responsible for the Marchi reaction (see p. 441) given by degenerating nerve fibres at this stage. Recent work by Morris, Hudson and Weddell suggests that the degradation of myelin occurs within the cytoplasm of the Schwann cells and that macrophages derived from external sources play little part in this process.

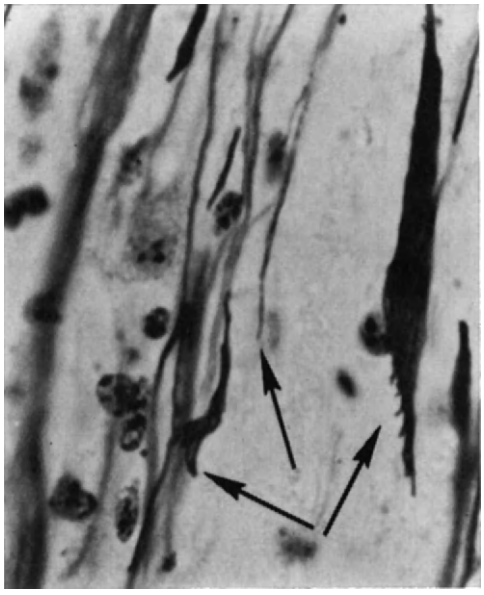


FIG. 11.14A. A micrograph of the growing tips (arrowed) of a group of regenerating nerve fibres. Silver impregnation. Preparation provided by Dr. A. Ostberg.

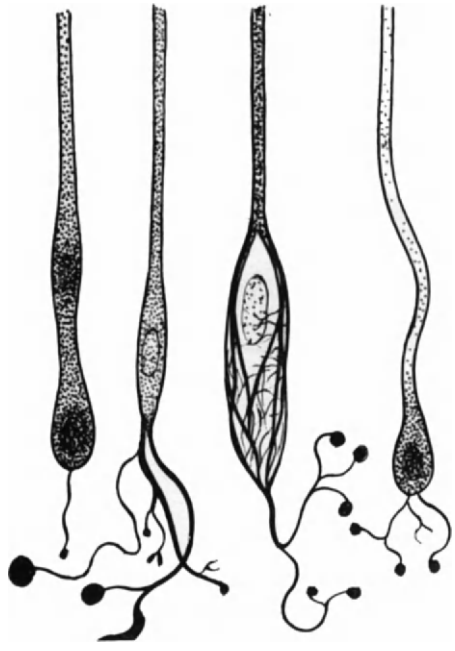


FIG. 11.14B. A diagram of some of the appearances often shown by the growing tip of regenerating nerve fibres. It is usual for several fibres to sprout from each axon.

Regeneration. Repair of the nerve fibres begins on the central side of the lesion almost immediately after the injury, and consists always of the outgrowth of new nerve processes. Within twenty-four hours the central stump of the axon has become swollen and is covered by proliferating Schwann cells. Many fibrils with bulbous endings sprout out from each axon (Fig. 11.14) and, unless the scar tissue is too dense, they penetrate through the wounded tissue to grow into the endoneurial tubes that are replacing the distal parts of the cut nerve fibres. Many of the sprouts spread into surrounding connective tissue and ultimately disappear: those sprouts that reach the tubes continue to grow onward, guided by them ultimately to establish connection with the original end organs. Many fibres may enter one tube, but one increases in diameter, becomes surrounded by Schwann cells and then myelinated: the other fibres atrophy. The growing tip clings to the Schwann cells, and ultimately becomes enclosed within their cytoplasm.

If regeneration fails, the central end of the cut fibre and the cell of origin both atrophy from disuse. Within the central nervous system no significant regeneration occurs although axonal

outgrowths occur in an abnormal fashion. It seems that axonal growth is prevented by the presence of scar tissue.

Degeneration and regeneration of non-myelinated nerve fibres differs from that of myelinated fibres only by the absence of the myelin sheath.

The rate of regeneration of nerve fibres after injury has been studied in the rabbit. Depending upon the nature of the injury, the growth rate varied from 3·5 mm. to 4·5 mm/day: furthermore, over the distances of regeneration studied the growth rate appeared to be remarkably constant, in this respect differing from other growth and regeneration processes. If the two cut ends are in apposition regeneration is facilitated: if there is displacement by trauma or scar tissue, suturing of the cut ends will facilitate repair, but this may never be functionally complete.

(*b*) Pathological Conditions

The nervous elements of nerve trunks may undergo changes in various pathological conditions: as all the tissues of the nerve are involved these are considered later (see p. 164).

C. NERVE ENDINGS

Nerves end either on some other neuron or in some peripheral organ such as skeletal muscle; this ending may be in the form of a free, unmodified termination of the axon or as a specialized ending which is adapted to serve a special function. Sensory terminations respond to stimuli in the periphery and transmit the nerve impulse to the central nervous system (CNS) whereas motor or effector endings pass on a nerve impulse from the CNS to the tissues that are to

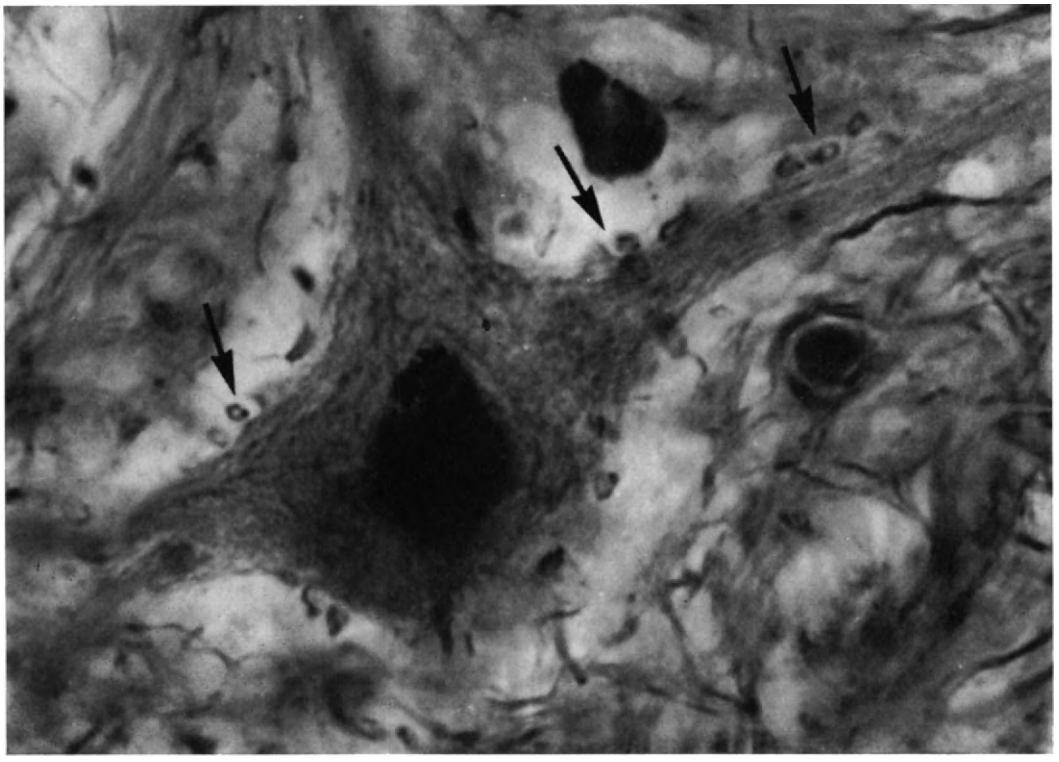


FIG. 11.15. A photograph of the "ring" appearance of "boutons terminaux" on a large motor neuron, seen in a silver impregnation technique. Preparation loaned by Dr. M. Fillenz.

5 μm.

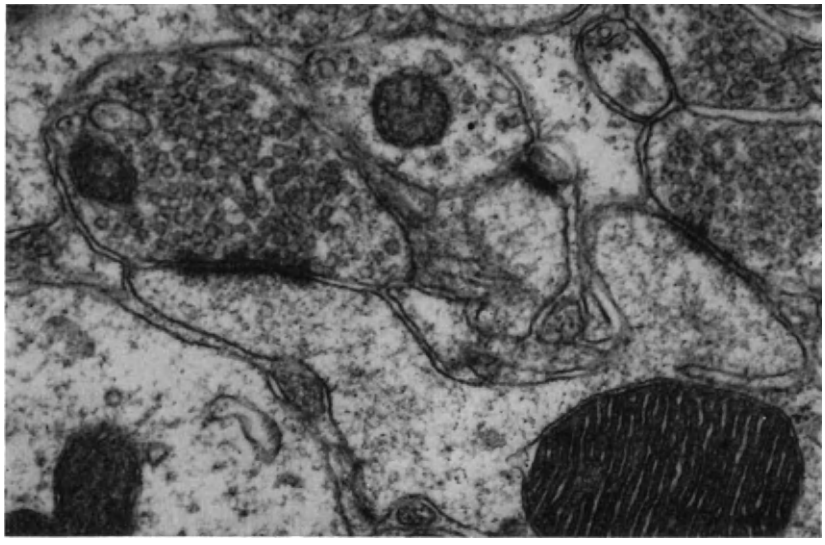


FIG. 11.16A. An electron micrograph of synapses in the central nervous system. Micrograph by Dr. G. Raisman and Pauline Field. 0·4 μm.

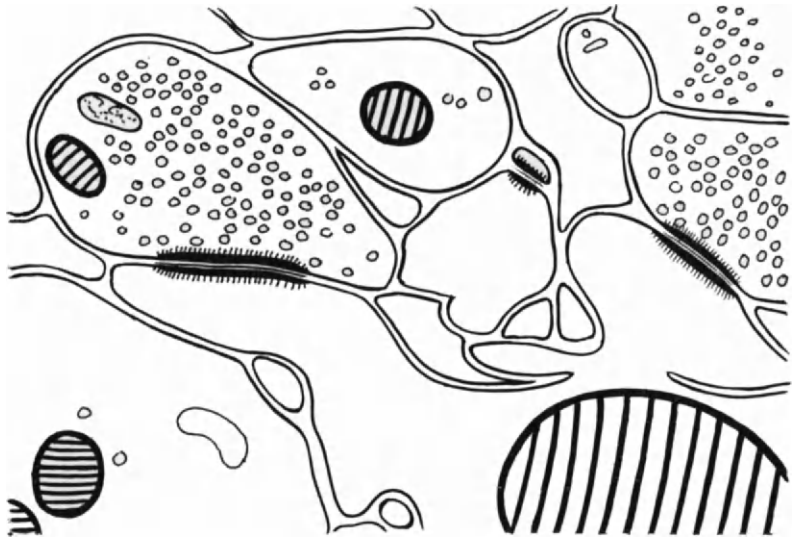


FIG. 11.16B. A diagram of the micrograph of 11.16A. The large cross-hatched structures are mitochondria, the synaptic thickenings are shown in heavy black and the synaptic vesicles in open circles.

respond. The specialized endings often attain a very high degree of complexity and textbooks of neuro-anatomy should be consulted for details. Only the commonest and simplest of the specialized nerve endings will be described briefly here.

(1) The Synapse

The synapse is that point of contact between the axon termination of one nerve cell and the dendrite or perikaryon of another. It is across this point of contact that the nerve impulse is transmitted from one neuron to the next. In the majority of cases the nerve terminals appear

swollen and silver impregnation methods show them as rings or sometimes knobs, which are usually called "boutons terminaux" (Fig. 11.15).

It is known that there is no cytoplasmic continuity between the two neurons at the synapse. E/M studies have shown a very clear and characteristic structure at the synapse (Fig. 11.16). The nerve terminal is bounded by a unit membrane which shows marked *presynaptic thickening* opposite the area of contact with the other neuron; here there is an area of *postsynaptic thickening*. Between the two cell membranes is the usual homogeneous layer of material which

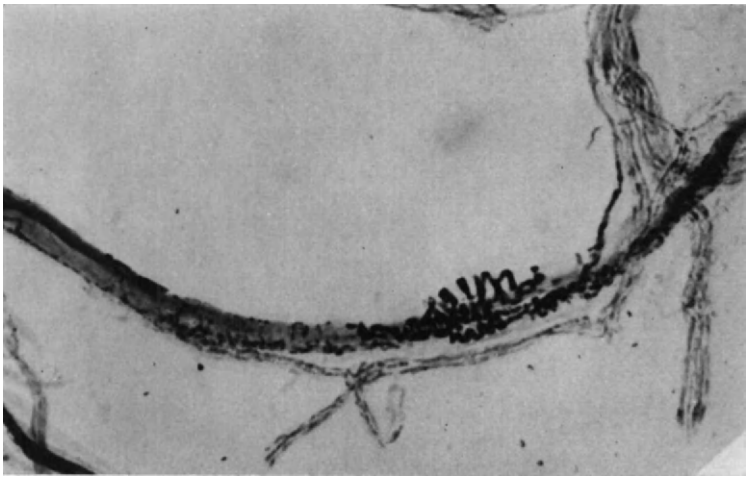


FIG. 11.17. A silver impregnation of a single muscle spindle teased from an extraocular muscle. The annulo-spiral nerve ending around the spindle is clearly shown. Preparation by Dr. A. Lockett.

120 μm.

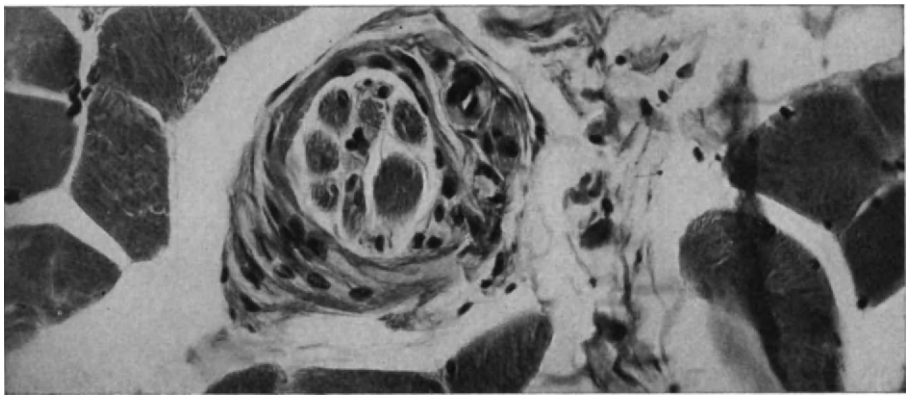


FIG. 11.18. Transverse section of a muscle spindle. The difference in size between the intrafusal fibres of the spindle and the ordinary skeletal muscle fibres is well shown.

30 μm.

resembles that of the basal lamina. The synaptic termination contains mitochondria, possibly neurofilaments and large numbers of small (40–60 nm.) membrane-bound vesicles. These are the so-called *synaptic vesicles*, each of which is thought to contain a quantum of the transmitter substances, e.g. acetyl choline and catecholamines. These, when liberated at the synapse, cause the propagation of the nerve impulse from one neuron to another although the exact manner in which the chemicals affect the post synaptic membrane to cause its depolarization is not yet clear.

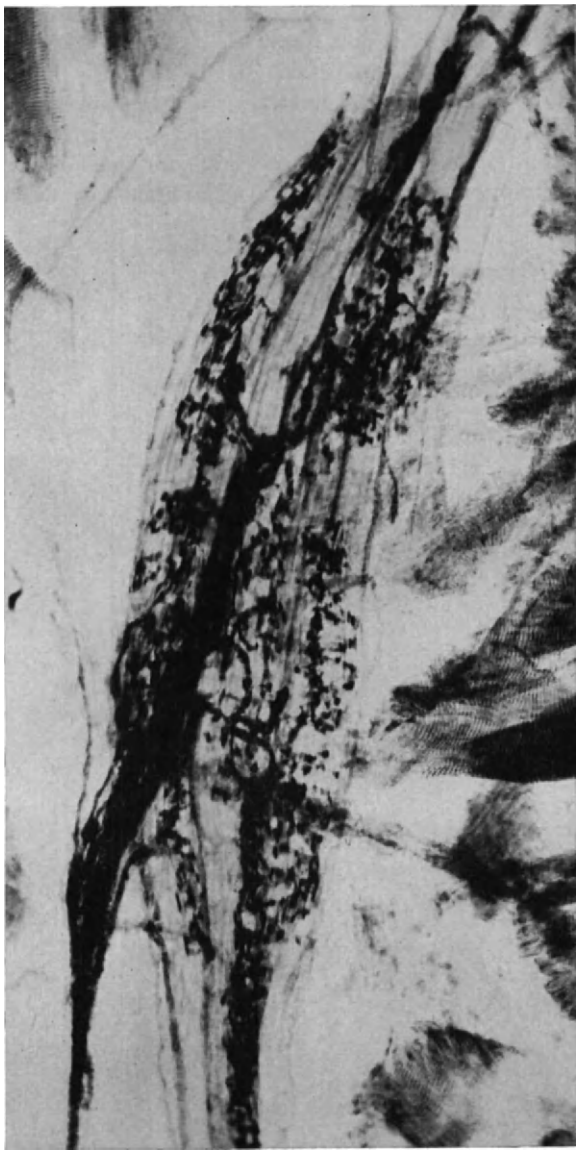


FIG. 11.19. A teased preparation of a tendon organ from an extensor muscle of the cat's forelimb. 100 μm.

(2) Sensory Nerve Endings

The nerve fibre is the dendrite of a neuron and the ending involves the arborization of its axon. Sensory nerve endings are either free or embody a complex organ. Free nerve endings are probably concerned primarily with the sensation of pain and possibly of light touch. Among the encapsulated organs, the Pacinian corpuscles are stimulated by stretch or deep pressure, as also are the neuromuscular spindles and tendon organs, whilst the plexuses around hair roots are sensitive to displacement of the hair by tactile stimuli. In addition some nerve endings—the chemoreceptors—respond to chemical stimulation. Other complex sensory endings have been described (e.g. the corpuscles of Meissner and Ruffini and the end-organs

of Krause). At various times specific sensory modalities have been ascribed to these; for example the Ruffini corpuscle was regarded as mediating the sensation of warmth whilst the Krause end-organ was responsible for the sensation of cold. More recent work, however, has thrown doubt upon this. Interested readers should consult the recent book by D. Sinclair for further details.

(*a*) **In Muscle and Tendon.** The nerve fibres may end either within the muscular or tendinous tissue or in the connective tissue between the fibres. Receptor nerve endings in striped muscle may consist simply of a branching and anastomosing net closely encircling the muscle fibre, or of the more complex *neuromuscular spindle*. The spindle is a cylindrical structure elongated in the direction of the long axis of the muscle fibres, and consists of from two to twelve thin intrafusal, striated muscle fibres, several nerve fibres of which one at least is a thick sensory

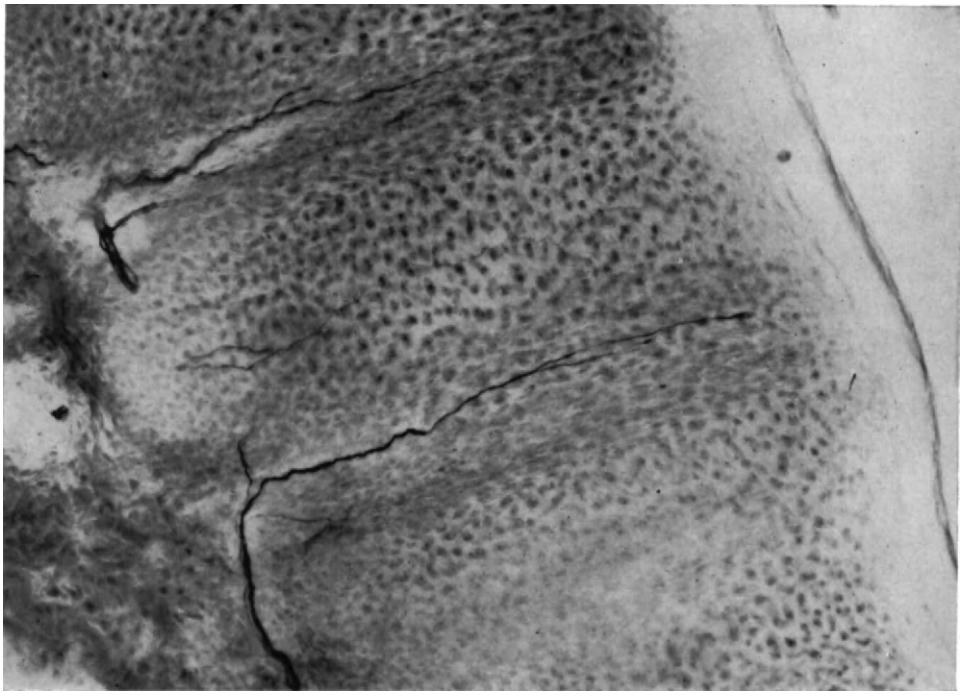


FIG. 11.20. Sensory nerve fibres ending in the epidermis of the skin. The surface of the skin is on the right of the picture.

fibre, blood vessels and connective tissue, all enclosed in a connective tissue sheath (see Figs. 11.17, 11.18). The nerve fibres end within the spindle by a series of rings or spirals (the *annulo-spiral endings*) wrapping round the individual fibres and by terminal arborizations (or *flower-spray endings*). The spindle may reach a length of 7 mm. It possesses also thin motor fibres, the γ fibres, that end in typical motor end plates (see p. 160). Both the annulo-spiral endings and the flower-spray endings are acting as stretch receptors, whereas the γ fibres are motor; they serve to set the sensitivity of the whole stretch receptor organ.

At the junction of muscle and tendon another complicated type of ending is often found, the *tendon organ of Golgi*. The tendon organ is spindle-shaped and includes several tendon bundles: one or more thick nerve fibres enter and break up into complex terminal arborizations on the tendon bundles (see Fig. 11.19).

Muscle spindles and the tendon organs act by forming the receptor end of a reflex arc. This

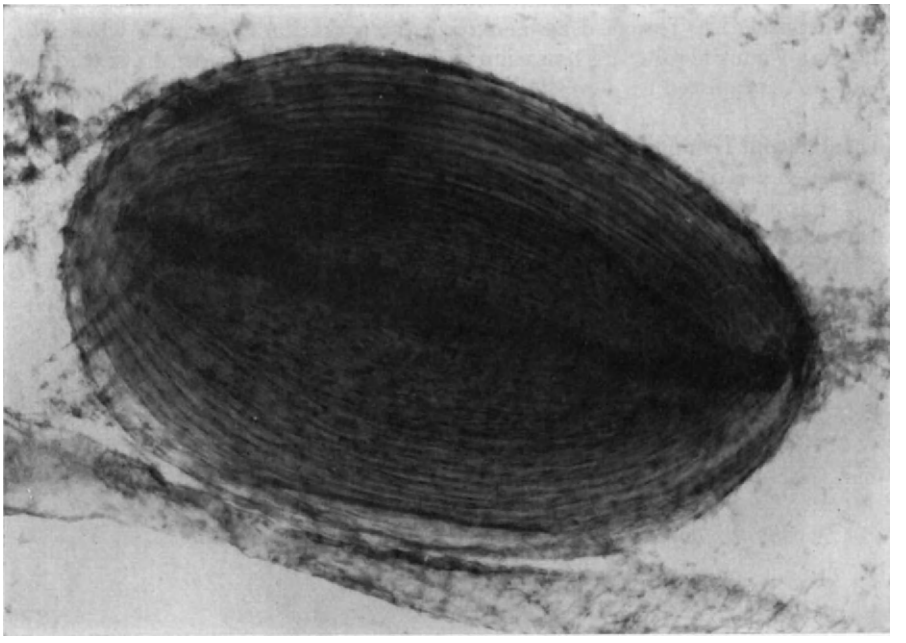


FIG. 11.21A. A whole mount of a Pacinian corpuscle in the mesentery. The nerve fibre runs up into the central core, which is surrounded by the many-layered capsule. 0·1 mm.

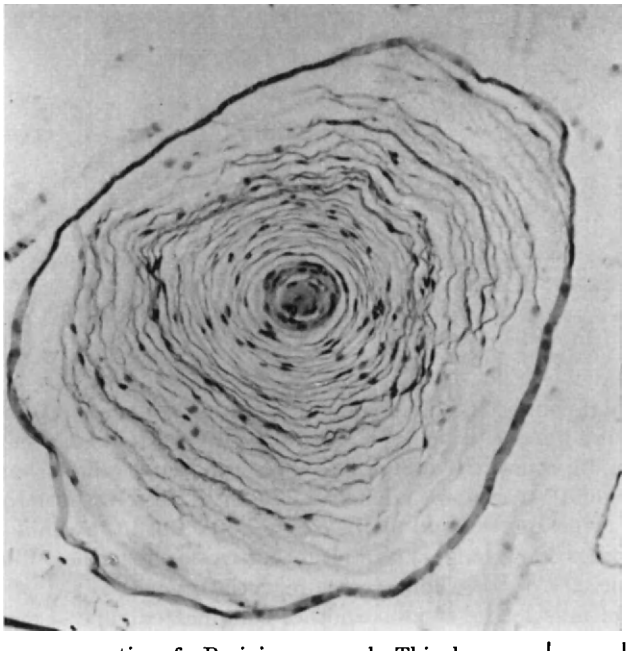


FIG. 11.21B. A transverse section of a Pacinian corpuscle. This shows the concentric layers of the capsule very well. 0·1 mm.

feeds back and activates the skeletal muscle which controls the constant postural adjustments taking place all the time.

In the connective tissue between muscle and tendon bundles are found both free terminal nerve arborizations, and also the encapsulated endings of Pacinian corpuscles (see below).

Receptor nerve endings of the free and of the spindle type are found in smooth muscle: in cardiac muscle complex terminal ramifications and structures like tendon organs have been described beneath the epicardium and endocardium, but not actually within the muscle substance.

(*b*) **In Epithelial Tissues.** There is usually a somewhat coarse, primary nerve plexus from which arise finer fibres that make a secondary plexus immediately beneath the epithelium. From the secondary plexus arise fine non-myelinated fibres that end by ramifying between the epithelial cells (Fig. 11.20); the actual endings are usually varicose. Sometimes, as in a stratified

FIG. 11.22. Motor end plates on skeletal muscle fibres of the tongue. Silver impregnation. 20 μm.

epithelium, some of the epithelial cells are modified to lie in relation to a terminal expansion of the axon: these are the tactile discs.

(*c*) **In Connective Tissues.** Connective tissue all over the body is very richly supplied with sensory nerve endings. These endings are of most varied form and may be either free or encapsulated. Free terminal arborizations, often with a small knob at the end, and free nerve nets are of very wide-spread distribution. Of the encapsulated type with a special connective tissue capsule the Pacinian corpuscles (corpuscles of Vater-Pacini) are the commonest (Fig. 11.21). Each corpuscle is supplied with one or more thick myelinated nerve fibres that then lose their myelin, and whose Schwann cell layer is continuous with the capsule. The capsule consists of many concentric lamellae of connective tissue lined by flat connective tissue cells arranged like the coats of an onion; the whole structure is often 1 mm. or more in length and is easily seen by the unaided eye. Any stretching of the connective tissue components of these corpuscles stimulates the terminal arborization of the nerve fibre. This type of nerve ending is of widespread distribution.

(3) Effector Nerve Endings

The nerve fibre is the axon of a nerve cell, which may be either of a "motor" cell of the spinal cord, a cell of a cerebral nucleus of an efferent cranial nerve, or a cell of an autonomic ganglion.

(*a*) **In Striated (Voluntary) Muscle.** The nerve fibres are mostly large myelinated fibres that terminate in specialized *motor end-plates* (Figs. 11.22, 11.23). The nerve fibre usually branches several times near its ending, each branch passing to a different muscle fibre; any one motor neuron, together with all those muscle fibres which it innervates is termed a *motor unit*. Such a motor unit may have only a few muscle fibres (as in the extra-ocular muscles) or very many (as in the large muscles which act as prime movers of the skeleton, e.g. gluteus maximus).

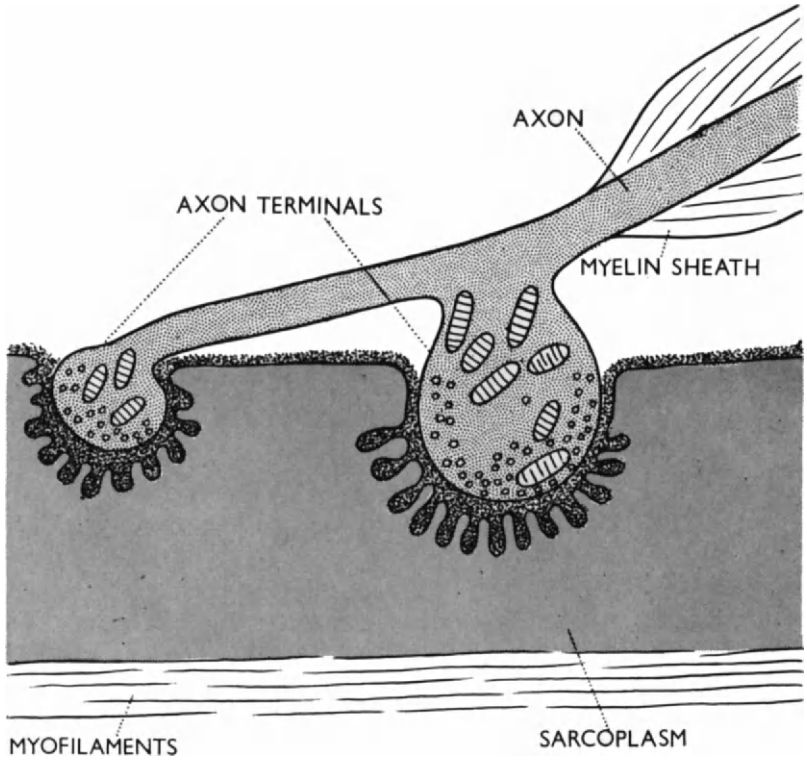


FIG. 11.23. A simplified diagram of the organization of a motor end plate. The basal lamina is shown in heavy stipple, completely filling the subneural clefts. Mitochondria and synaptic vesicles are present in the axon terminals.

Any one muscle fibre has only one end-plate except in the case of tongue muscles and the muscle fibres in the neuromuscular spindles, both of which often have two endings. The endoneurium joins the sarcolemma: the myelin sheath is lost on reaching the muscle fibre, and the sheath of Schwann cells spreads over the surface of the sarcolemma and disappears, and the naked axon comes into contact with the sarcoplasm and breaks up into terminal ramifications. The terminal branches of the axon lie in troughs in the sarcoplasm of the muscle fibre and here the axonal and sarcoplasmic membranes are closely applied to each other, separated by a homogeneous protein/mucopolysaccharide complex similar to that of the basal lamina. The axoplasmic terminals show large numbers of vesicles similar to the synaptic vesicles in the axo-dendritic synapses of the CNS. The layer of sarcoplasm just beneath the axonal terminals

is known as the *sole plate* and is characterized by an accumulation of muscle nuclei in its substance. There is also a corrugation of the sarcolemma which forms the so-called *subneural clefts* (Fig. 11.23). It is here that histochemical techniques have demonstrated high concentrations of the enzyme *acetyl cholinesterase*.

(*b*) **In Cardiac Muscle.** The nerve fibres are non-myelinated: they branch repeatedly and end in free varicose thickenings.

(*c*) **In Smooth Muscle.** The nerve fibres are non-myelinated: they form networks from which arise fine fibres that branch to penetrate between and sometimes into the muscle cells, and finally these end in small varicose thickenings.

(*d*) **In Glands.** The nerve fibres are chiefly non-myelinated: outside the basal lamina of the gland cells they make a network from which some fibres penetrate to make a second network on its inner surface. The final endings are usually slender varicose fibres between the glandular cells.

Reaction of Nerve Endings to Section of the Nerve Fibre

The nervous components of a nerve ending always degenerate completely if the nerve fibre is cut. In the case of the motor end plate the protoplasmic "sole" of sarcoplasm persists for a year or more, although the nervous component of the ending disappears. If regeneration of the nerve fibre is accomplished, a new functional nervous arborization is formed in relation to the surviving components of the original structure. If denervation has been prolonged, the re-innervation of old end-plates is much diminished, and new end-plates may be formed where the nerve fibres touch the sarcoplasm.

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CHAPTER 12

PERIPHERAL NERVES, GANGLIA, NEUROGLIA AND MENINGES

PERIPHERAL NERVES

Outside the central nervous system the nerve fibres are bound together into bundles by means of connective tissue to form the peripheral nerves. A transverse section of a nerve (Fig. 12.1) shows that it consists, as a rule, of a number of smaller bundles or funiculi consisting of the nerve fibres; when a nerve trunk divides some of the funiculi are found running in each of the resulting nerves.

The connective tissue surrounding the whole nerve and connecting the funiculi is the *epineurium*: it contains blood vessels, lymphatics, fat cells and sometimes nerve fibres (nervi nervorum) for the coats of the nerve. The collagen and elastic fibres are arranged chiefly in a longitudinal direction, and the usual connective tissue cells are present.

Each funiculus is, in turn, closely invested with dense connective tissue arranged in concentric layers, termed the *perineurium*. Between the connective tissue layers lymph clefts are present, conveying lymph from the funiculus to the lymphatics of the epineurium. From the

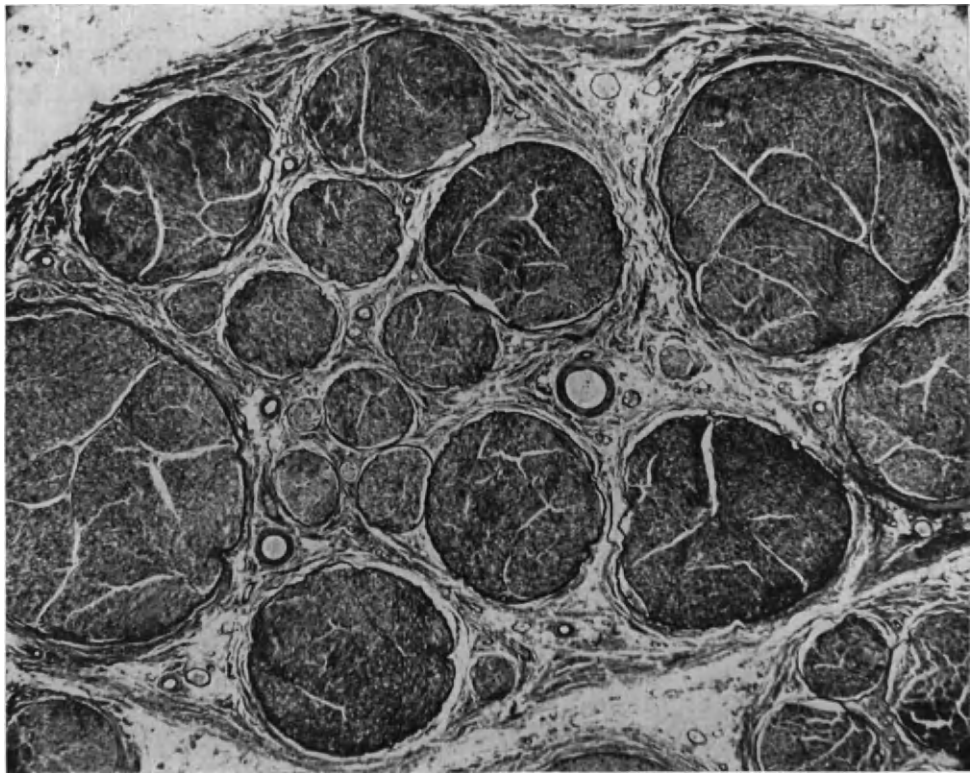

FIG. 12.1. A transverse section of part of the sciatic nerve. Many nerve funiculi may be seen, each surrounded by perineurium. The epineurium binding the funiculi together into a single nerve contains many blood vessels.

perineurium fine strands of connective tissue fibres and cells, together with blood capillaries, pass into the funiculus into the spaces between the separate nerve fibres: this is the *endoneurium*. The nerve fibres may be nearly all myelinated, or mostly non-myelinated: the relative proportions vary very greatly.

In peripheral nerves three distinct size ranges of fibres can be seen (Fig. 12.2). The large fibres (1–22 μm. diameter) are known as type A fibres; these are mainly motor but with the addition of some sensory fibres. Type B fibres are the visceral sensory fibres. These are below 3 μm. in diameter, whilst the smallest fibres (forming the group C fibres) are unmyelinated and of 1·2–0·4 μm. in diameter; these are almost entirely autonomic and sensory, transmitting pain

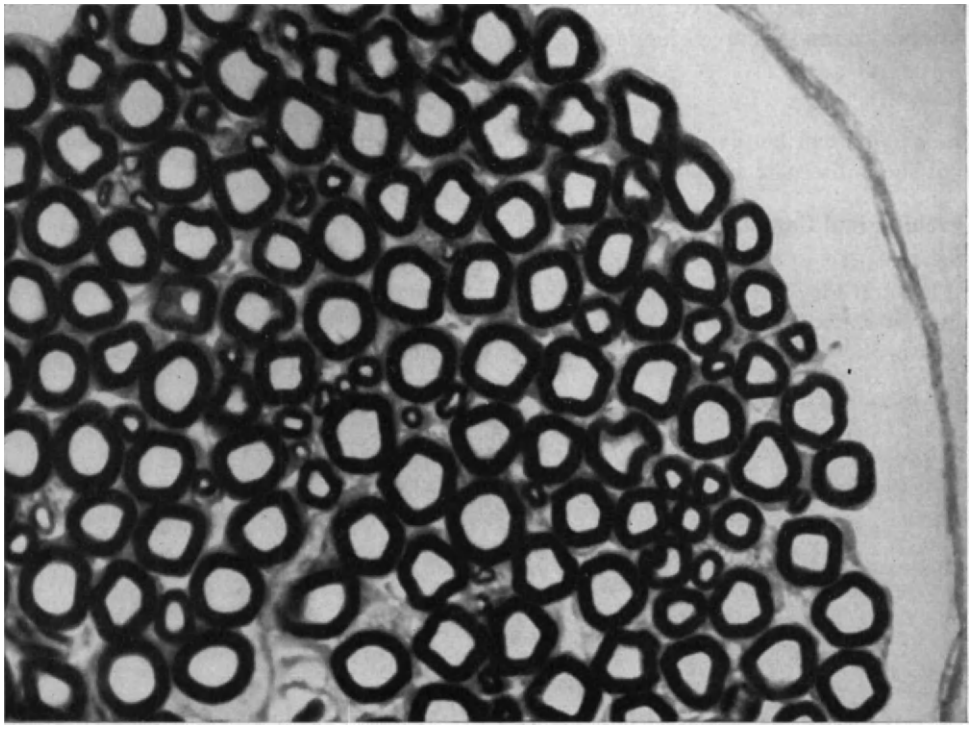

FIG. 12.2. A high power optical micrograph of part of the sural nerve. The myelin sheaths of the nerve fibres have been rendered dark by treatment with osmium tetroxide. Note the differing sizes of the nerve fibres.

sensations. Type A fibres conduct the fastest, at 15–100 metres per second, type B are intermediate in speed, ranging from 3–14 metres per second, whilst the type C fibres conduct the slow impulses at 0·5–2 metres per second.

Functional differences (e.g. regarding the spike durations, the thresholds and the after-potentials) can be distinguished by neurophysiological methods.

As the fibres in the nerve root pass through the pia mater, the peripheral fibres become invested with connective tissue (endoneurium and perineurium): as they pass through the dura mater the bundles are further bound together by epineurium.

As the nerve trunk divides into smaller branches the connective tissue sheath becomes thinner. Ultimately the epineurium disappears and the perineurium is hardly distinguishable from endoneurium, and is represented by a thin fibrous membrane covered with flat connective tissue cells. From this membrane extend the delicate connective tissue filaments that wrap

round each nerve fibre as the neurilemma: this is closely adherent to the Schwann cell sheath (see p. 145).

Reactions of Nerves to Various Conditions

Nerve trunks are affected by a variety of pathological conditions. The somewhat vague term of neuritis includes diverse affections: broadly speaking, there are two classes of such change. In the first group the essential change is a primary degeneration of the nerve fibres with little reaction of the connective tissues: it is frequently due to poisons (such as lead, alcohol or bacterial toxins) in the blood: in this case the nerve fibres undergo degenerative changes similar to those following trauma. In the second group the primary change is in the connective tissue of the nerve and is usually of an inflammatory nature: any changes in the nerve fibres themselves are secondary.

GANGLIA

The ganglia can be divided into those belonging to the afferent cerebrospinal nerves and those of the autonomic system.

(*a*) Cerebrospinal Ganglia

The majority of the nerve cells are large* and spherical, of the pseudo-unipolar type (see p. 139 and Fig. 11.1): the single process usually acquires a myelin sheath quite close to the cell, and at a node of Ranvier the process divides by a T-shaped branching. One of these divisions is the dendrite from the periphery, the other is the axon passing into the central

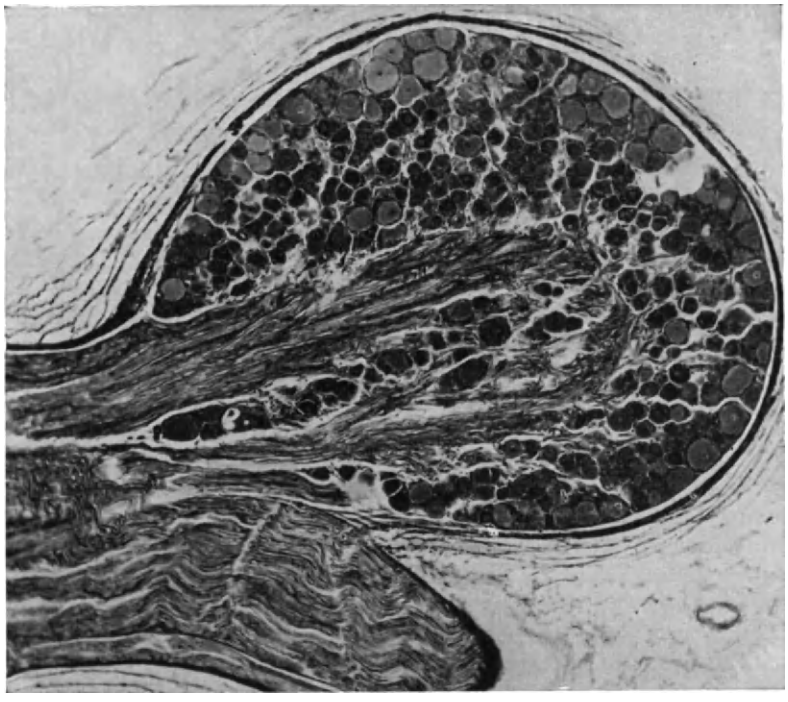

FIG. 12.3. A section through a human spinal ganglion. The marked peripheral arrangement of the nerve cells is clearly shown. 40 μ.

* As nerve cells are relatively large, the plane of an ordinary section does not necessarily pass through the nucleus: such a cell then *appears* to have no nucleus.

nervous system. Sometimes, particularly in old age, other short bulbous processes may arise from the cell and end within its capsule.

Each nerve cell within the ganglion is surrounded by a capsule. There is a layer of so-called satellite cells, corresponding to neuroglia, that closely invests the cell and is continuous with the layer of Schwann cells of similar origin that covers the processes. Outside this capsule is another very delicate connective tissue covering, derived from the endoneurium, and thus in continuity with the neurilemma of the nerve fibre. Between these two capsules is a capillary network and minute lymphatics.

In the spinal ganglia the cells are gathered in groups, chiefly at the periphery of the ganglion (Fig. 12.3), and the fibres, nearly all of which are myelinated, are also gathered in groups. This arrangement renders these ganglia easy to distinguish from those of the autonomic system.

The whole ganglion is enclosed in a dense connective tissue capsule from which is derived the fine connective tissue that pervades the whole ganglion. This ganglion capsule is continuous with the epineurium and perineurium of its associated nerve trunks.

(*b*) Autonomic Ganglia

The autonomic ganglia do not show such a definite internal arrangement of cells and fibres as is seen in the spinal ganglia. The nerve cells are usually small, with extremely well-

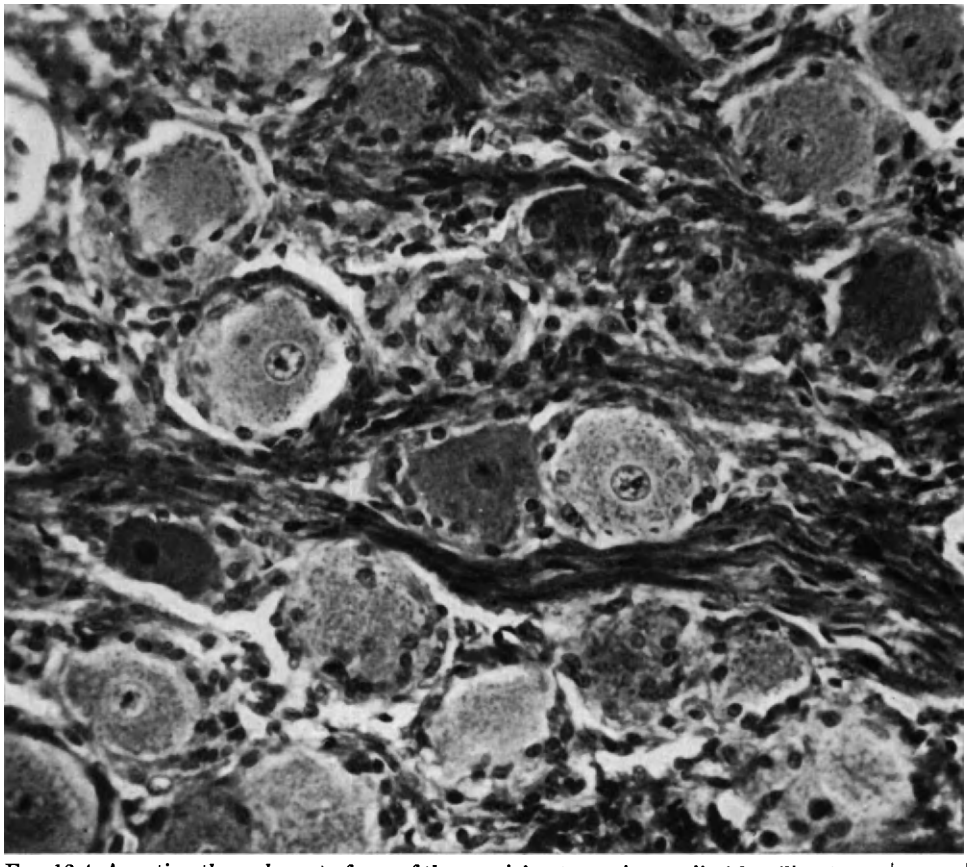

FIG. 12.4. A section through part of one of the cranial autonomic ganglia (the ciliary). Note the interlacing arrangement of the nerve cells and fibres. The large neurons are surrounded by numerous nuclei which belong to the satellite cells.

20 μm.

defined Nissl granules; the cells are usually multipolar and the axon is generally non-myelinated. Much evidence is now available which suggests that the neuronal cytoplasm is very active in the synthesis of the catecholamines which act as the neurotransmitter substances for this type of cell. These are synthesized in the cell body and subsequently they pass down the axon to the nerve terminal, where they are released. The E/M has shown that they appear as vesicles, with electron-dense "cores". Should the axon be ligated, the dense-cored vesicles tend to accumulate proximal to the constriction. A somewhat ill-defined capsule of satellite cells surrounds each nerve cell in the ganglia of the sympathetic chain, and the arrangement of the connective tissue elements of the whole ganglion is the same as in the spinal ganglia. Some of the dendrites of the cell sometimes remain within the cell capsule.

In the outlying ganglia the definite capsule of satellite cells is sometimes replaced by an interlacing network of small, branching, spindle-shaped cells, probably of similar origin.

There is no definite grouping of cells or of fibres in the autonomic ganglion, and as most of the nerve fibres are non-myelinated the appearance of the ganglion in a section (Fig. 12.4) is quite distinct from the characteristic picture given by a spinal ganglion.

Development of the Ganglia and Nerves

(*a*) Ganglia

The spinal ganglia are derived from the neural crest cells, the original neuroblast cell becoming transformed into the pseudo-unipolar type: the neuroglial elements are ultimately arranged as capsule cells for the nerve cells, and are continuous with the lemmal covering of the outgrowing nerve processes. The ordinary connective tissue elements are provided by the surrounding mesoderm.

The autonomic ganglia are derived partly from cells that have migrated from the neural crests, and partly from cells that have migrated out from the primitive spinal cord with the anterior root fibres: both neuroblasts and neuroglial elements are provided in this way.

(*b*) Nerves

Each of the nerve fibres which compose a nerve is developed from a neuroblast cell. The efferent root fibres grow out as axons of neuroblast cells of the more ventral part of the primitive spinal cord, rupture the external limiting membrane and emerge from the antero-lateral part of the cord: dendrites of these cells appear later. The afferent root fibres grow out from neuroblast cells of the neural crests. In each case the Schwann cell sheath of the nerve fibre is provided by cells from the neural crest, or occasionally by cells that migrate from the neural tube with the emerging nerve fibres: these cells are similar to neuroglia and also to the satellite cells of the nerve ganglia, all being ectodermal in origin. The myelin sheath is formed from the cell membranes of the Schwann cells (Fig. 11.9).

Thus in the nerve the Schwann cells covering the fibres represent the neuroglial component, while the epi-, peri- and endoneurium are ordinary connective tissue of mesodermal origin.

NEUROGLIA

The nervous system consists primarily of neurons, but these are supported and held together by a special type of tissue known as *neuroglia*: connective tissue proper in the nervous system is limited to that accompanying blood vessels and to the meninges (p. 170).

Neuroglia proper, like the ependyma cells, develops from the primitive epithelium of the neural tube (p. 137): the ependyma can be regarded as neuroglial cells that have retained their primitive shape and position. As the migrating spongioblasts differentiate, some of them develop processes which become attached to blood vessels: they thus become more or less fixed. Other developing neuroglia cells also give off processes which interlace with the free processes of these attached cells, and so provide a supporting meshwork to maintain the neurons in place.

Any stained section of the central nervous system shows large numbers of nuclei and fibres scattered between the nerve cells and nerve processes: this is the neuropil tissue that fills up all the spaces. The detailed structure can, however, be made out only by the aid of special methods. "Neuroglial tissue" includes the ependyma, neuroglia proper, and the Schwann and capsular (satellite) cells of the peripheral nervous system: the latter two cell types have been already described (pp. 145, 165). In addition to the neuroglia proper of neuroectodermal origin, the tissue of the central nervous system is also pervaded with another type of glial tissue, the *microglia* or mesoglia of Hortega: this is of mesodermal origin. The microglial cells migrate into the brain substance just before birth from the surrounding pia mater, chiefly in the neighbourhood of the choroid plexuses.

A. Neuroglia Proper

Three kinds of neuroglial cell can be distinguished, protoplasmic astrocytes, fibrous astrocytes and oligodendrocytes.

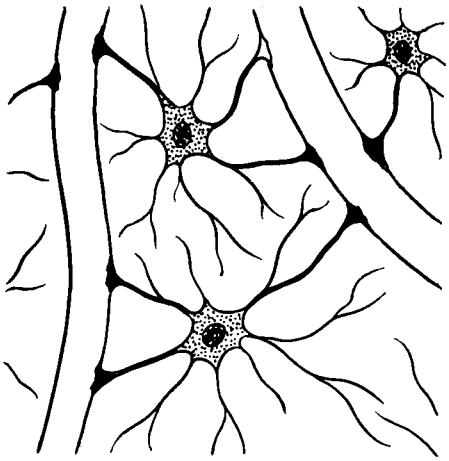

FIG. 12.5. A diagram of protoplasmic astrocytes in the grey matter of the CNS. Some of the cell processes forming the vascular end feet are attached to the capillaries, which they completely invest.

(1) Protoplasmic Astrocytes (Figs. 12.5, 12.6)

These are found most abundantly in the grey matter of the CNS. The cells have large ovoid nuclei, granular cytoplasm and many branching, somewhat coarse, protoplasmic processes: some of the large branches are attached to small blood vessels by enlarged "vascular end feet" or pedicles. The cells contain no fibrils. Some of the smaller cells of this type are closely applied to nerve cells; the hypothesis of Ramon y Cajal that nerve cells are invested with neuroglia except at those sites where they are in synaptic contact with other neurons has received support from recent E/M studies. Astrocytes may easily be recognized in electron micrographs of the CNS by their characteristic voluminous cytoplasm of very low electron density.

(2) Fibrous Astrocytes (Fig. 12.7)

These are found chiefly in the white matter of the CNS between the bundles of myelinated nerve fibres. The cells contain thick, unbranched fibrils that extend for long distances and branch outside the cells. The fibres differ from those of connective tissue and possibly consist of α-keratin. These cells also are often attached to capillaries by vascular end feet.

FIG. 12.6. Protoplasmic astrocytes in the grey matter of the CNS revealed by silver impregnation. Notice the large number of cell processes of this type of glial cell. 20 μm.

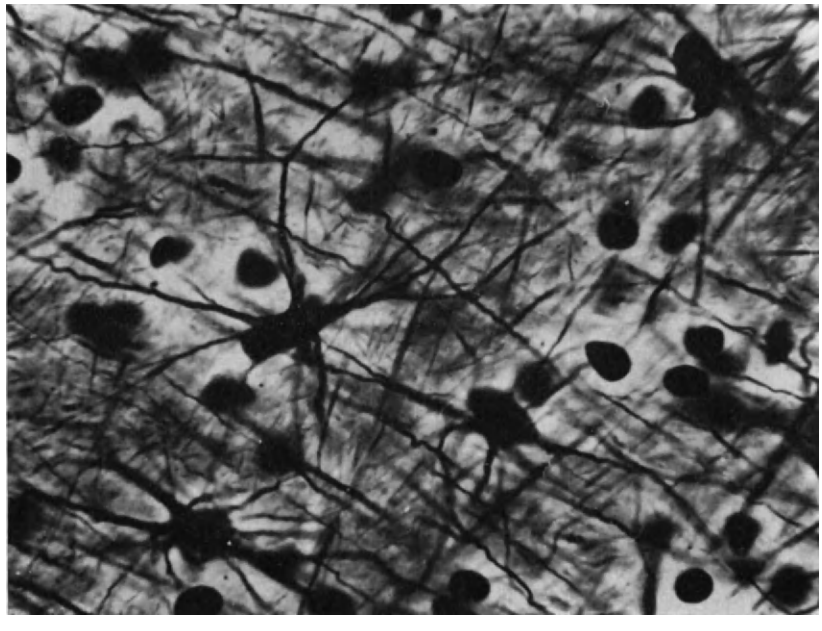

FIG. 12.7. A micrograph of fibrous astrocytes in the white matter of the CNS. Silver impregnation. 20 μm.

(3) Oligodendrocytes (Fig. 12.8)

These are found in the white matter in rows between the myelinated nerve fibres, and in the grey matter closely applied to nerve cells or blood vessels. The cells and nuclei are small and stain darkly; they have very few processes, and these are fine, hardly branched at all, and have no vascular attachments.

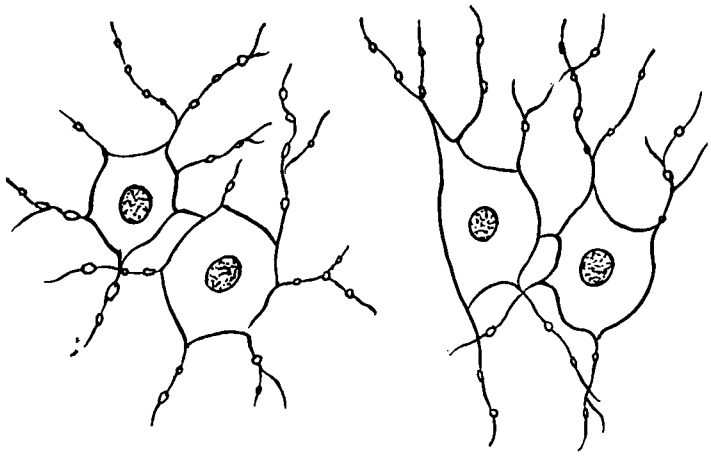

FIG. 12.8. A diagram of the typical morphology of oligodendroglia in the white matter of the cerebrum (after Rio del Hortega).

Functions of the Neuroglia Proper

The astrocyte is the true supporting structure of the nervous system, providing an extremely complicated framework of cells and fibres in which the neurons are suspended and insulated from one another: the cells probably help also to nourish the neurons. By virtue of their vascular end feet, which completely surround the capillaries, the neuroglia may provide an important anatomical element in the formation of the blood-brain barrier. The oligodendroglia helps in the formation and maintenance of myelin in the CNS, and may enable a neuron to maintain functional activity over its whole length. Fibrous glia is particularly abundant in the spinal nucleus of the fifth cranial nerve, the superior olives, and the periventricular grey matter: it is scanty in the deeper part of the cerebral cortex, caudate nucleus and putamen.

B. Microglia (Fig. 12.9)

These cells are more numerous in grey matter than in white. They have very small, elongated cell bodies, with deeply staining nuclei: from each end of the cell arises a thick process

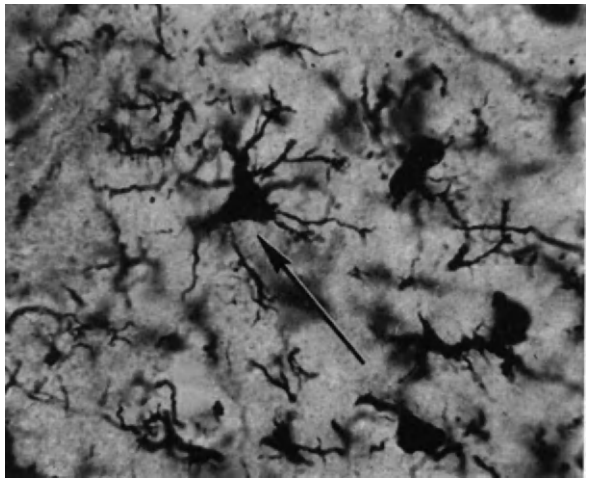

FIG. 12.9. Microglia (arrowed) demonstrated by a silver technique.

that branches freely and is beset with spinous projections. These cells are characterized in the E/M by their very electron-dense cytoplasm, in which occasional phagocytosed material may be seen.

Functions of the Microglia

The microglia cells are phagocytic and function as macrophages for the central nervous system: they represent therefore essentially a defence mechanism and may be considered as part of the reticulo-endothelial system. They are of mesodermal origin.

Reactions of the Glial Tissue to Various Conditions

All elements of the glial tissues, as distinct from the neurons, retain the power of proliferation. Glial elements are the chief source of tumours of the CNS.

Glia responds rapidly to injury or to toxic conditions, although it is not quickly affected by a degree of oxygen lack that is sufficient to damage nerve cells. The response is either of the nature of rapid death or of hypertrophy (gliosis): the astrocytes are particularly concerned in the formation of scar tissue. The microglia displays the usual activities of macrophages, phagocytosing cellular débris and eliminating toxic products of catabolism. These cells can migrate to the seat of the lesion, being attracted chemotactically: the cell-form may change under these conditions, the cells usually losing their processes and becoming rounded and amoeboid during active migration.

MENINGES

The brain and the spinal cord are covered by three membranes, the *meninges*; the outer is called the *dura mater* because of its dense, firm character, the middle, more delicate and loosely arranged covering is known as the *arachnoid membrane*, whilst the inner membrane, closely applied to the CNS, is called the *pia mater*. Between the outer two membranes is a very narrow subdural space, and between the arachnoid layer of the inner covering and the pia mater is the subarachnoid space containing cerebrospinal fluid (C.S.F.). This subarachnoid space communicates with the ventricular system (also containing C.S.F.) by means of the median and lateral foramina (foramina of Magendie and of Luschka).

Dura Mater

In the spinal canal the dura is separated from the vertebral periosteum by a wide epidural space containing loose connective tissue, fat and many veins: it is firmly attached to the spinal cord by the denticulate ligaments. In the cranial cavity the dura serves as the periosteum of the internal surface of the cranial bones to which it adheres loosely: at the sutures and at the base of the skull it is attached firmly. The dura of the brain consists of an outer layer that is richer in cells and blood vessels than the inner layer with its greater quantity of collagen bundles: the whole membrane is relatively poorly vascularized.

The inner layer forms large free folds that project into the cranial cavity and divide it into compartments. The most important of these folds are the *falx cerebri*, hanging into the median fissure between the cerebral hemispheres as a sickle-shaped partition, the *tentorium cerebelli* stretching across the superior surface of the cerebellum between it and the occipital lobes of the cerebrum, and the smaller *falx cerebelli*. Along the cranial lines of attachment of these septa the two layers of the dura are widely separated and enclose the dural venous sinuses (Fig. 12.10).

Everywhere the inner surface of the dura is covered with a layer of flat mesothelial cells.

Arachnoid Membrane and Pia Mater

The pia mater and the arachnoid membrane develop as a single sheet from primitive mesenchyme: this sheet splits into two layers at the time that cerebrospinal fluid is first

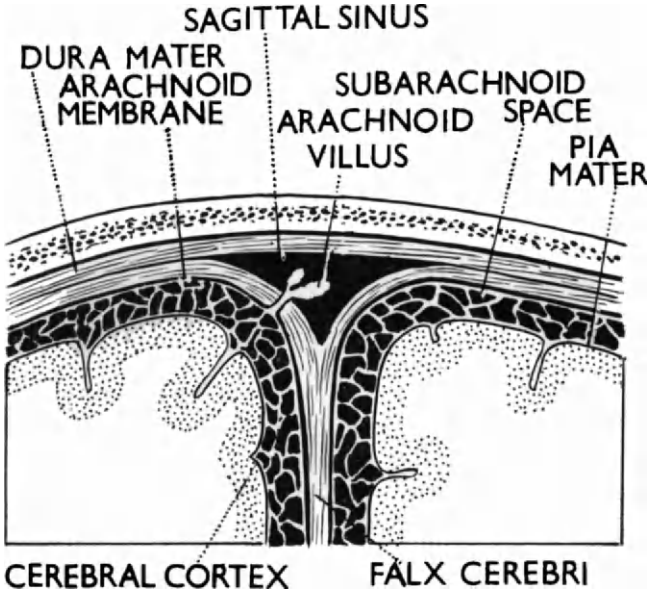

FIG. 12.10. A diagram of a coronal section through the meninges and part of the cerebral cortex to show the subarachnoid space, the dural venous sinus and an arachnoid granulation projecting into it (after Weed).

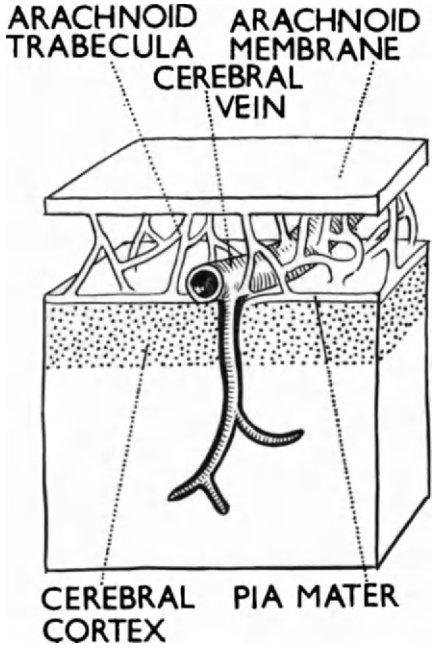

FIG. 12.11. A diagram to show the relationship of the subarachnoid space to the arachnoid and pia mater (after Weed).

formed, some of the fluid being found in the subarachnoid space formed by the split. The membranes of brain and spinal cord resemble one another in structure.

The arachnoid is a very thin membrane of fine connective tissue, especially rich in reticular fibres, lying next to the dura mater; its smooth outer side of squamous cells is covered by a film of serous fluid. It bridges over all the sulci on the surface of the brain and spinal cord, forming subarachnoid spaces of varying extent: from its inner surface pass innumerable fine trabeculae crossing the sub-arachnoid space and connecting the arachnoid with the pia mater (Fig. 12.11). The latter membrane also consists of fine connective tissue, rich in reticular and elastic fibres which form a closely felted network, but it differs from the arachnoid in containing blood vessels and adhering closely to the surface of the brain, dipping down into the sulci. Over the convolutions the subarachnoid space is narrow, in the sulci it is deep: in certain places in the brain it is greatly enlarged into the cisternae, of which the cisterna magna is the most important. Both membranes contain fibroblasts and macrophages, which are particularly numerous along the blood vessels: mast cells and lymphocytes also occur, together with many cells of embryonic type.

Both surfaces of the arachnoid, the trabeculae and the outer surface of the pia, are covered with a layer of squamous cells, probably a mesothelium.

Over the convex portions of the brain the arachnoid membrane is prolonged into villi or tufts that project through the dura into the lacunae communicating with the dural sinuses. The cavity of the arachnoid villus, in communication with the subarachnoid space, contains C.S.F.: here, therefore, the C.S.F. is separated from the blood in the sinus only by the very thin mesothelial membrane (see Fig. 12.10). This is the main pathway for outflow of C.S.F. into the venous circulation. In middle age some of the villi become enlarged, thickened and even calcified, and are known as *Pacchionian corpuscles* (*granulations*).

Recent studies of Shantha and Bourne have shown that the membranes of the pia and arachnoid are continued along the cranial and spinal nerves. These authors regard these layers, which they term the *perineural epithelium*, as an active diffusion barrier between the neural tissue and the surrounding environment. In addition, they accompany all the blood vessels that enter to supply the nervous tissue: here the layers are more or less fused and blend with the adventitial coat of the vessels.

Nerves of the Meninges

The blood vessels of the meninges are supplied by rich nerve plexuses of sympathetic origin, the fine terminal fibres penetrating the media coat of the vessels. Simple terminal arborizations of sensory type are found in the adventitial coat. The connective tissue of the dura also contains nerve plexuses with terminations like Meissner's corpuscles.

Lymphatics of the Meninges

The central nervous system and the meninges possess no true lymphatics. There is a network of lymphatic capillaries outside the dura, and this is in communication with cleft-like perineural spaces within the sheaths of the cranial nerve roots, particularly the olfactory, optic and auditory nerves. These clefts (except the optic) are not in communication with the subarachnoid space. The clefts in the adventitial coats of the blood vessels of brain and meninges drain into the subarachnoid space.

Choroid Plexuses (Fig. 12.12)

In certain parts of the brain the roof plate remains undifferentiated as thin, non-nervous layer. The pia mater covering this becomes highly vascularized and differentiated, throwing the structure into tufts. These choroid plexuses are found in the roof of the third and fourth ventricles, and in part of the wall of the two lateral ventricles. The choroidal villi are folded and invaginated into the ventricles of the brain so that a large surface is exposed to the

ventricular fluid. The covering epithelial cells are at first ciliated like the rest of the ependymal cells; in later life the cilia are lost and the cells appear cubical, with a spherical nucleus, and often contain vacuoles or fat; the E/M shows them to bear large numbers of microvilli on their luminal surfaces. These microvilli are unusual in that they are often expanded into a bulbous swelling at their tips. The capillaries of the choroid plexus have been shown by electron microscopy to differ in their structure from those in the remainder of the CNS in that they possess very thin endothelial cells which have large fenestrae. These are presumably adaptations for the easy transudation of the plasma of the blood which is going to constitute the C.S.F. The pial core of the villi contains large capillaries and venous sinuses, and in the perivascular tissue are many macrophages: in adult life concretions are often present.

Nerve fibres are said to end in relation to the epithelial cells.

Reactions of the Meninges to Various Conditions

The dura mater is relatively non-vascular and is not readily involved in infective processes. The pia-arachnoid membrane, with its rich blood supply, reacts on the other hand to many and varying conditions. It can be shown by intra-vital staining methods that the walls of the blood vessels of the pia provide a barrier against the passage of foreign material in the general circulation. If there is inflammation of the meninges (meningitis), or the presence of some foreign substance in the cerebrospinal fluid, the macrophages, and also according to some workers the mesothelial lining cells, round off and mobilize as polyblasts and free macrophages:

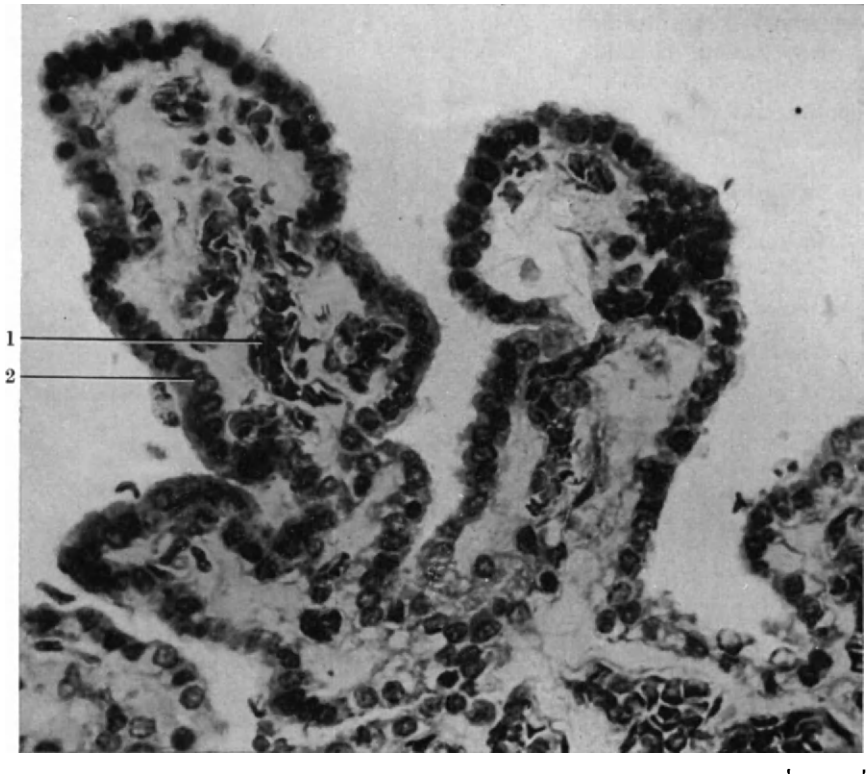

FIG. 12.12. A micrograph of a section through the choroid plexus of a full-term human foetus.

1, A large capillary in the pial core of the villus.
2, Epithelial cells with basal nuclei.

they are found in the subarachnoid space in the cerebrospinal fluid, together with an exudation of plasma and of granular leucocytes from the blood.

GENERAL STRUCTURE OF ORGANS

The various tissues described in the preceding chapters are not as a rule found separately, but are associated with one another to form an *organ* for some particular function. In almost every case there is a framework of connective tissue with all its constituent elements: this sometimes makes a capsule, often with trabeculae, that penetrate into the substance of the organ. The connective tissue component is usually spoken of as the *interstitial tissue* (or *stroma*) and the specific tissue of the organ as the *parenchyma*. Further, every organ has its own blood supply, its lymphatic supply and its nerve supply, so that all these structures are also present.

When examining the minute structure of an organ, it should be remembered always that variations will be seen that depend on such factors as the state of activity or of rest of the organ, the age of the individual, the diet, the temperature, and so forth. The aim of the student should be to recognize variations that fall within physiological limits in order to be able later on to detect whether the conditions seen fall outside these limits and within the realm of pathological change. The two series of changes often overlap.

In the following chapters the organs are considered in the following order:

Reference for further detailed study:

M. L. Barr, "The Human Nervous System", Harper and Row (1972).
G. H. Bourne (Editor), "Structure and Function of Nervous Tissue", Vol. 1, Academic Press (1968).
S. W. Kuffler, "Neuroglial Cells", *Proc. Roy. Soc. B*, **168**, 1–21 (1967).
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W. F. Windle (Editor), "Biology of Neuroglia", Thomas (1958).

CHAPTER 13

THE BLOOD CIRCULATORY SYSTEM

The system responsible for the circulation of the blood consists of a closed system of tubes of varying calibre; one part of the original tube is specially modified to become the central pump or heart.

It is important to remember that the *whole* system—heart, arteries, capillaries, veins—is lined by a flattened endothelium of mesodermal origin, which rests on a varying amount of connective tissue. In the capillaries this constitutes the whole wall, but in larger vessels and in the heart thickenings of the wall are developed, composed of connective tissue elements (collagen and elastic fibres and membranes), and muscle fibres (smooth in the vessels, striated in the heart).

The variations in relative amounts and disposition of these various tissues in the different vessels is closely allied to the particular functions of those vessels.

ARTERIES

The arteries may be divided into three types:

(1) Elastic arteries—large vessels, with walls in which *elastic* elements predominate.

(2) Muscular arteries—medium-sized and small vessels, with a predominance of *muscular* elements in their walls.

(3) Arterioles—small muscular vessels, immediately controlling the supply of blood to the capillary bed, and whose walls consist almost entirely of circularly-arranged muscle fibres. Transitional stages are found where an artery of one type passes over into one of the next type.

In the wall of any artery three layers can be distinguished (Fig. 13.1).

(*a*) **Tunica intima,** or internal layer. This is always lined by an endothelium composed of squamous cells, and the sub-endothelial elements are oriented in a direction longitudinal to the vessel. It is separated from the middle coat by a fenestrated membrane, the *internal elastic lamina.*

(*b*) **Tunica media,** or middle layer. This is the thickest layer and the one on which depends the character of the artery. Its elements are arranged in a direction circular to the vessel, and it is often separated from the outer coat by a membrane known as the *external elastic lamina.*

(*c*) **Tunica adventitia,** or outermost layer. This coat gradually merges with the loose connective tissue accompanying the vessel: its elements run for the most part longitudinally. The strength of the arterial wall depends chiefly on the collagen fibres of this outermost coat, which prevent undue distension or rupture.

The wall of an artery is relatively thick and not affected by changes of external pressure. Contraction of the circular muscle cannot completely occlude the lumen because of the existence of an elastic lamina internal to the muscle: it is for this reason that arteries remain patent at death and cross-sections of fixed preparations (in which the muscle is contracted) show the distinctive scalloped line of internal elastic lamina with the characteristic corrugation of the intima coat (Fig. 13.3), as the elastic membranes are unable to contract and are thrown into longitudinal folds; the endothelial nuclei consequently tend to bulge into the lumen.

(1) Elastic Arteries

(The aorta, carotids, subclavian, axillary and iliac arteries.) In this category of vessels the wall is very resilient and elastic and not very thick relative to the size of lumen when compared

with the muscular arteries. Only part of the energy of the contraction of the heart goes into the circulation of the blood, the remainder being utilized in distending the walls of the elastic arteries. During diastole of the heart the elastic recoil of these vessel walls then provides the kinetic energy which helps to maintain the propulsion of the blood and smooths out its flow. This mechanism makes a continuous flow possible from an intermittent pump such as the heart.

(*a*) **Tunica Intima.** The endothelial cells are elongated in the direction of the long axis of the vessel. The subendothelial layer consists of a small amount of ordinary loose connective tissue and then a layer of connective tissue containing many fine elastic fibres. These gradually merge into the fenestrated membrane of the internal elastic lamina which is not sharply

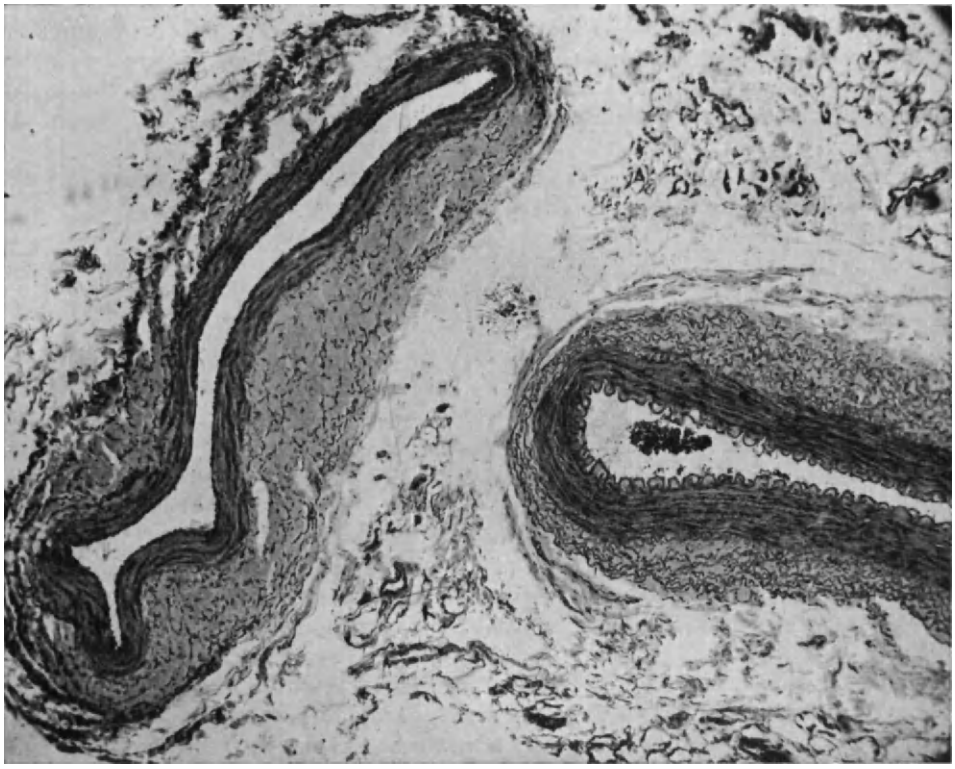

FIG. 13.1. A small artery and vein in transverse section. The vein (on the left) shows the darkly staining tunica media surrounded by the adventitia, whilst the artery shows the intima thrown into folds, the tunica media and the adventitia.

150 μm.

marked off from the elastic membranes in the tunica media of the artery. Near the boundary of the intima and the media are found longitudinally-running muscle fibres.

(*b*) **Tunica Media.** This coat consists chiefly of elastic tissue (Fig. 13.2), arranged circularly as discontinuous membranes that often have holes in them: these membranes may be about 2·5 μm. thick. They may take the form of interlacing and anastomosing elastic fibres. Between the elastic membranes are thin layers of loose connective tissue with collagen fibres and fibroblasts, and short smooth muscle cells: in this tissue lymph circulates freely.

(*c*) **Tunica Adventitia.** In large arteries this layer is relatively thin. Where it is in contact with the tunica media it contains longitudinally arranged elastic fibres; more externally the thin elastic fibres run circularly. The adventitial coat consists chiefly of bundles of collagen fibres which merge gradually with the surrounding areolar tissue.

(2) Muscular Arteries

The majority of the medium sized arteries belong to this group. In general, the smaller the vessel, the thicker is its wall.

(*a*) **Tunica Intima.** The endothelium is covered externally by a subendothelial layer which diminishes in thickness with the decreasing size of the artery. This layer consists of cellular connective tissue, with very fine elastic fibres and a few smooth muscle fibres. The internal elastic membrane is well marked (Fig. 13.3): in later life it tends to split into several layers.

(*b*) **Tunica Media.** This coat consists chiefly of smooth muscle, the fibres arranged circularly

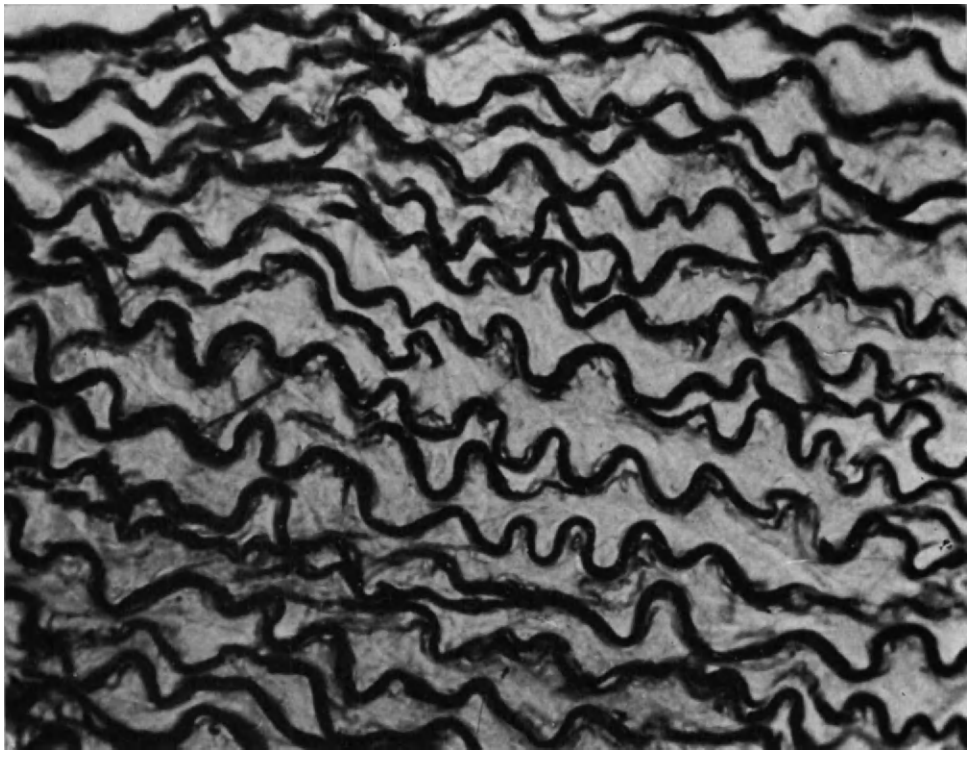

FIG. 13.2. A photomicrograph of part of the tunica media of the aorta. It has been stained with orcein which colours the elastic tissue (dark in the photo). Notice the characteristic post-mortem convolutions of the elastic fibres.

to the lumen of the vessel. The larger vessels possess fine elastic networks between the groups of muscle fibres and a small amount of connective tissue. The external elastic membrane divides this layer sharply from the outer coat.

(*c*) **Tunica Adventitia.** In this layer are found numbers of elastic fibres directed longitudinally or tangentially, together with collagen bundles. The outer part of the coat is very loose and merges into the surrounding connective tissue, this arrangement permitting of a certain amount of easy movement of the artery and alterations in the size of its lumen.

Changes in the degree of contraction and relaxation of the muscle cells in the wall of muscular arteries influences their resistance to the blood flow, so affecting the blood pressure; such changes in the calibre of the vessels also allows changes in the pattern of the distribution of blood to various organs.

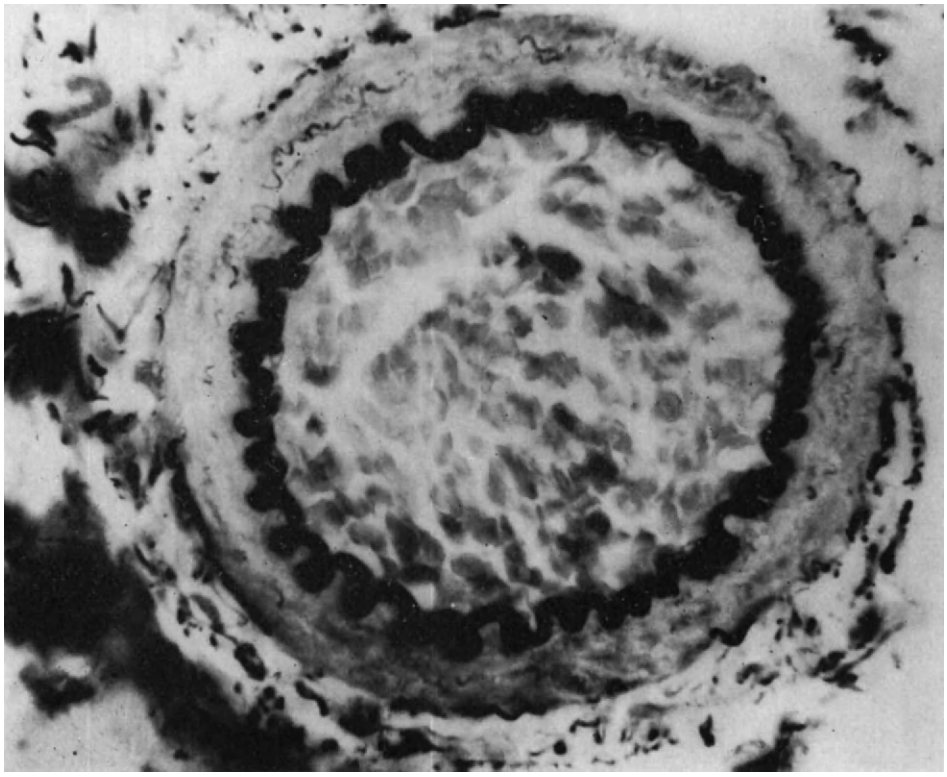

FIG. 13.3. Transverse section of a muscular artery stained to show the elastic tissue. The internal elastic lamina is prominent and shows the characteristic post-mortem corrugations; a few elastic fibres can be seen amongst the unstained muscle fibres of the tunica media and amongst the connective tissue of the adventitia. 15 μm.

(3) **Arterioles** (Fig. 13.4)

The larger arterioles possess the usual three coats. The tunica intima consists of endothelium separated by an extremely delicate connective tissue layer from the well-marked internal elastic lamina or fenestrated membrane. The tunica media consists of smooth muscle fibres wrapped round the intima, while the adventitial coat consists of loose connective tissue, with a few longitudinally arranged elastic and collagen fibres.

As the arterioles diminish in size the endothelium remains unchanged, but the internal elastic lamina becomes thinner and then disappears. The muscle fibres are progressively diminished in size and number and the adventitial coat is lost.

For details of the blood supply and the nerve supply of the walls of the arteries see pp. 187, 188.

VEINS

The general structure of the walls of the veins is similar to that of the arteries. The same three layers can usually be distinguished although they are not so clearly defined and the same elements enter into their constitution (Fig. 13.5). In general, the walls of the veins are very much thinner in proportion to the size of the lumen than are those of the arteries (Fig. 13.1); this enables them to be affected by changes of external pressure. The walls, although thin, are however very strong because the connective tissue components are greatly developed, whereas

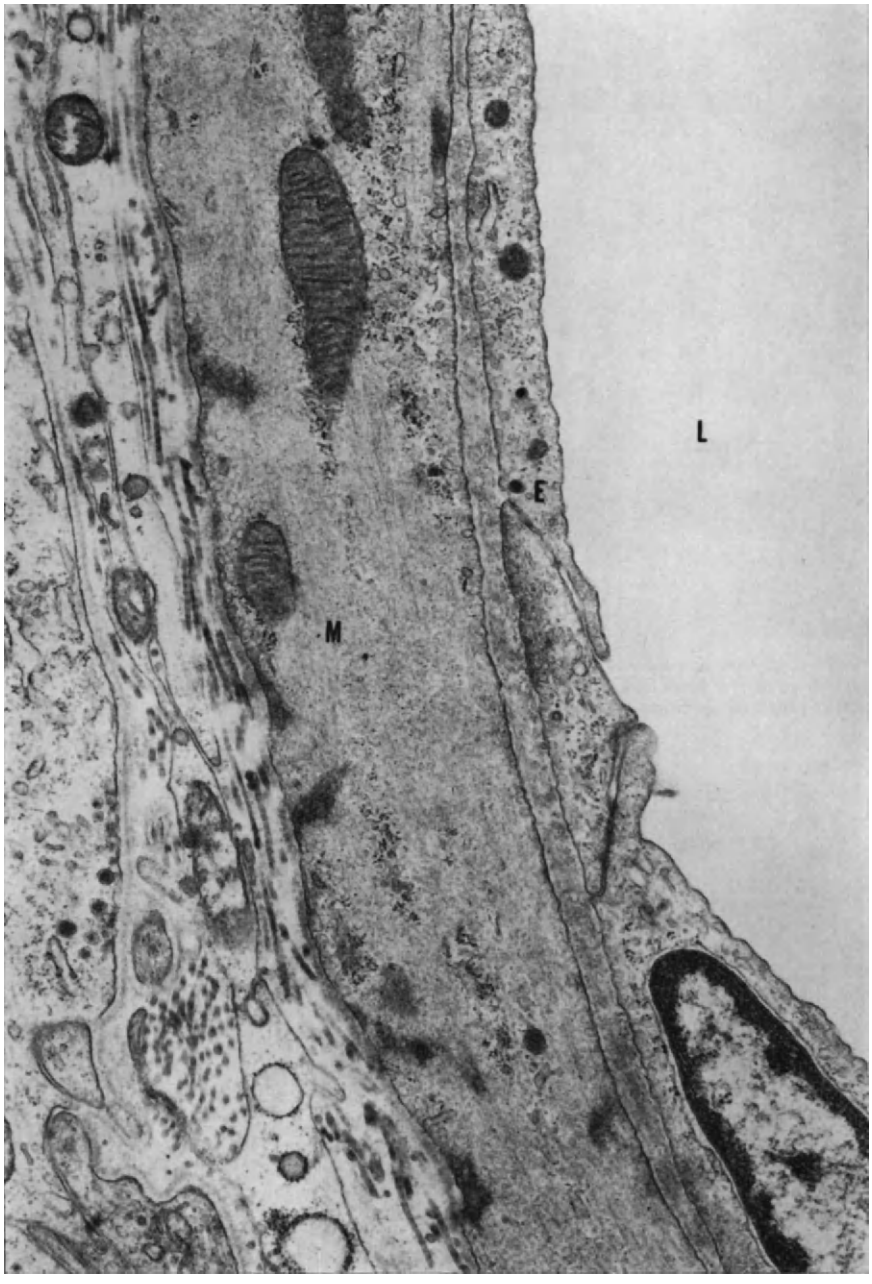

FIG. 13.4A. An electron micrograph of a small arteriole in section. The endothelium shows parts of three cells, one with its nucleus in the plane of section. The endothelium is surrounded externally by basal lamina and smooth muscle cells (M) seen here in cross section. Micrograph by courtesy of Dr. P. Field.

5 μm.

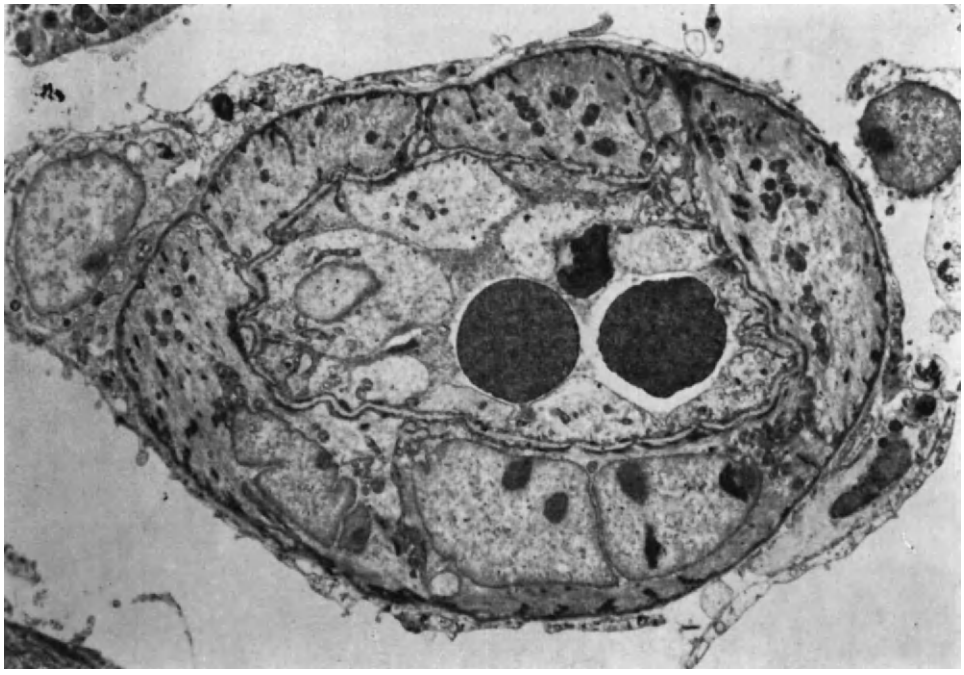

FIG. 13.4B. A similar arteriole in transverse section. Two red blood corpuscles occupy the centre of the vessel lumen. Two of the smooth muscle cells show their nuclei in this plane of section.

5 μm.

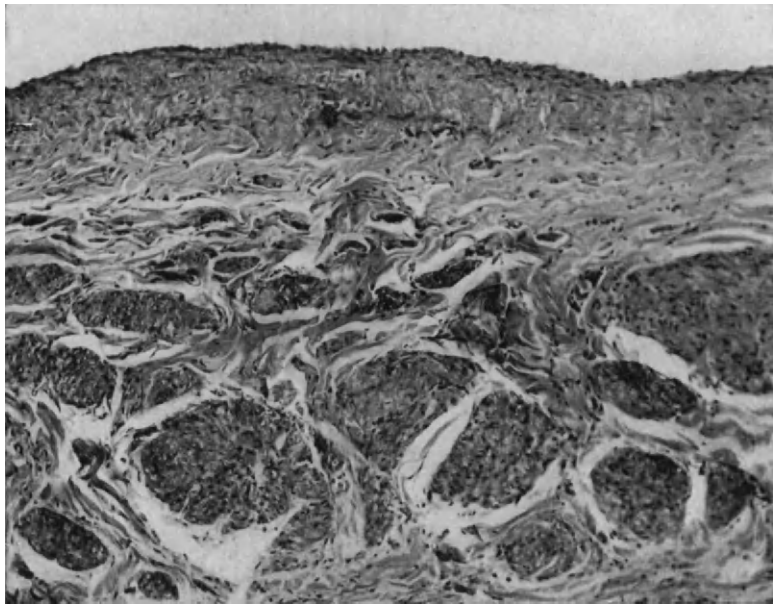

FIG. 13.5. A micrograph of a transverse section through the vena cava. Note that the tunica intima and tunica media are not sharply marked off from one another.

100 μm.

the muscular and elastic elements are relatively inconspicuous: in many veins the collagen fibres have a spiral arrangement. After death the walls tend to collapse instead of contracting like those of the arteries.

Tunica Intima. The endothelial cells are less elongated than those of the arteries. Externally there is a little connective tissue with a few fine elastic fibres. This layer merges into the tunica media.

Tunica Media. The middle coat is always relatively thin, and contains a little elastic tissue and muscle and a considerable amount of collagen fibres, which are continuous with those of the outer coat. It is thickest in the veins of the lower extremity.

Tunica Adventitia. Usually this coat is well developed, being much thicker than the middle coat. It consists of muscle and collagen and elastic fibres: the muscle fibres are chiefly longitudinal.

Valves of Veins

Valves are found in most veins over 2 mm. in diameter, and are particularly strong and numerous in the lower limbs: these valves prevent the blood in the veins from flowing back from the heart. They are semilunar, pocket-like flaps on the internal surface, with the free edge

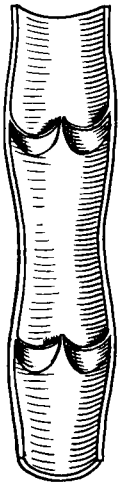

FIG. 13.6. A diagram of part of a vein slit open to show the arrangement of the valves. The direction of blood flow would be from the bottom to the top of the picture.

directed toward the heart (Fig. 13.6). They flatten out against the wall of the vein with the blood stream, and if the blood tries to flow back the pockets fill up and occlude the lumen of the vein. Between the valve and the wall of the vein is the sinus of the valve: the vessel wall is stretched and rather thin at this part. Valves are usually arranged in pairs opposite to one another.

The valve, covered by the endothelium of the vein, consists of connective tissue in which elastic fibres are well-developed on the side of the vein directed against the blood flow and absent on the sinus side.

Valves are not found on the course of veins within the cranium and vertebral canal, nor in the umbilical vein, the venae cavae, the pulmonary vein, nor in the veins of most of the viscera.

CAPILLARIES

The terminal ramifications of the small arterioles are usually connected with the corresponding terminal ramifications of the small venules by a capillary network: the transition is gradual

and the density of the network in any organ varies directly with the metabolic activity of that organ.

The arteriolar branches become progressively smaller and thinner-walled as the amount of blood carried and the internal pressure diminish. Ultimately the connective tissue coat is lost, and the muscle coat is either very poorly developed or absent, and only the endothelium remains: these vessels constitute the *capillary bed* (Fig. 13.7).

The distribution and arrangement of the capillary bed is not easily seen in tissues prepared by the normal techniques of histological sectioning and staining; if, however, a coloured mass of gelatin or Indian ink is injected into the vascular system and the tissue prepared in the form of a cleared wholemount, then the vascular pattern may be easily studied (Fig. 13.8). Alternatively, a plastic or latex injection medium may be used and the surrounding tissues then removed by macerating them away to leave a cast of the vascular bed. This type of *corrosion cast technique* can yield very instructive preparations.

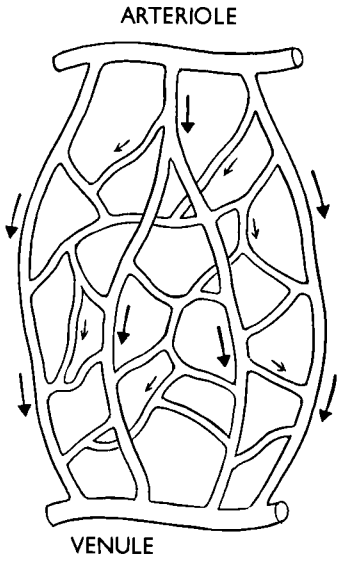

FIG. 13.7. A diagram of part of a capillary bed; the direction of blood flow is indicated by arrows.

In very small capillaries one single endothelial cell may form the wall of the tube, while in wider capillaries two or three curved cells may surround the lumen (Fig. 13.9). The average diameter of the capillary is about 8 μm., allowing of the passage of the blood corpuscles in single file (Fig. 13.10). Of these vessels many are completely closed for considerable periods when the tissue is in a resting condition: when active functioning begins many or all of them open up, and the blood circulates through them. A permanently open arteriovenous channel is, however, always present (Zweifach).

E/M studies have added a great deal to our knowledge of the capillaries. As a result of the recent work by Fawcett and others, capillaries may now be classified into the *muscular* (or continuous) type and the *fenestrated* type (Fig. 13.11). The former (found in smooth muscle, cardiac muscle, lung and other situations) show an uninterrupted endothelial cell of approximately equal thickness, with occasional large luminal bulges where the cell nuclei are situated (Fig. 13.12).

The cytoplasm of the muscular type of capillary endothelium is also characterized by the presence of numerous pinocytotic vesicles, 60–70 nm. in diameter, associated with the plasma membranes of both surfaces of the cell and also occurring free in the cytoplasm (Fig. 13.12). It has been suggested that these vesicles are largely responsible for the exchanges which take

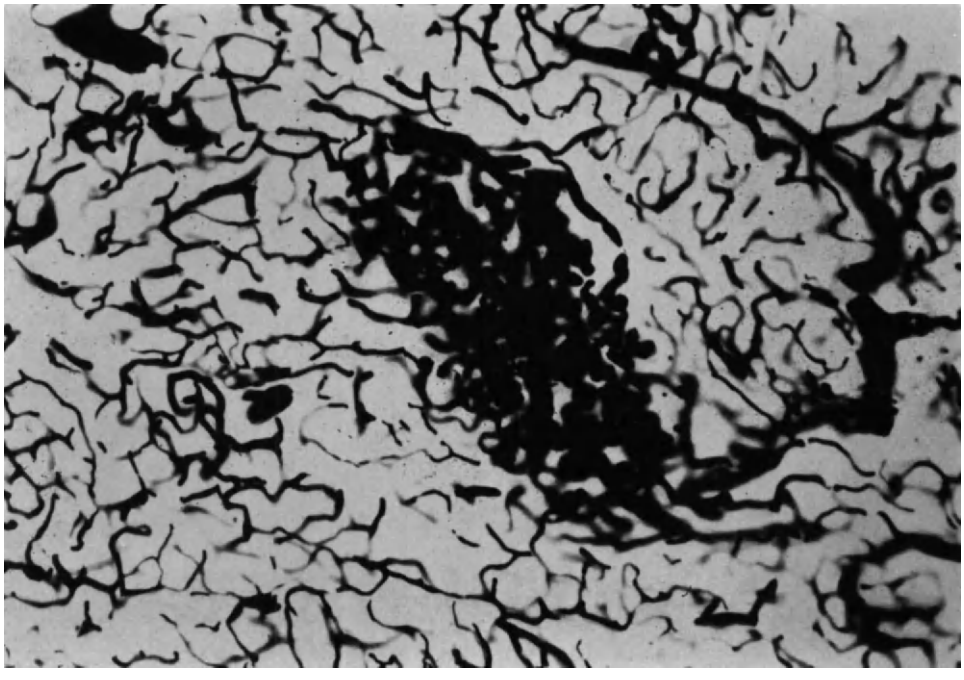

FIG. 13.8. A micrograph of part of a thick section of the pancreas in which the blood vessels have been injected with Indian ink. There is a great increase in the density of the capillary bed in the centre of the picture which corresponds to the site of an Islet of Langerhans.

FIG. 13.9. A diagram to show the fitting together of the endothelial cells to form the wall of a capillary.

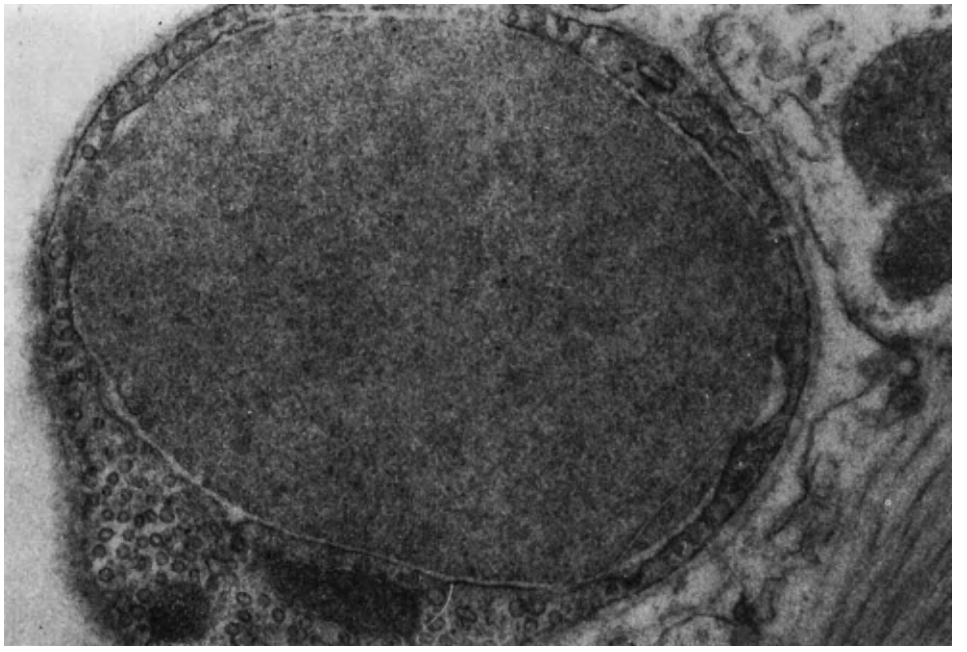

FIG. 13.10. An electron micrograph of a transverse section of a capillary. Note the thin endothelium with numerous pinocytotic vesicles (especially at the lower left). The endothelial cells are surrounded by the basal lamina and the lumen is occupied by a single red blood corpuscle. 0·75 μm.

place across the capillary endothelial cell wall; recent work by Karnovsky, however, using an electron-opaque marker substance has thrown doubt on this. He suggests that the bulk of the capillary transport takes place through the spaces between adjacent cells of the endothelium.

The capillary endothelial cells are typically surrounded by the continuous basal lamina of mucoprotein material. The fenestrated capillaries (Figs. 13.11, 13.13) occur in the renal glomerulus, in endocrine glands and in the intestinal villi. This type of capillary endothelium is characterized by the circular areas where the cytoplasm of the endothelial cell appears to be

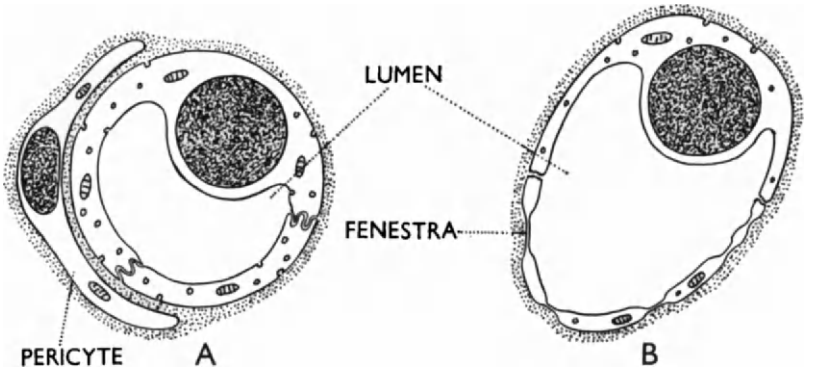

FIG. 13.11. Diagrams of sections through the two types of capillary recognized by Fawcett. A, muscular type; the endothelium is relatively thick with many pinocytotic vesicles. B, fenestrated type; note the thin areas—the "fenestrae".

absent. The fenestrae (about 80–100 nm. in diameter) are not completely open, except perhaps in the renal glomerulus, but are closed by a thin diaphragm.

It is suggested that these fenestrae occur where there must be a particularly active and unimpeded exchange between the tissue fluid and the blood.

As the capillary vessels originate from the embryonic connective tissue (see p. 191) they are accompanied throughout their course by connective tissue elements. These may be represented by perivascular cells (or *pericytes*, similar to cells described by Rouget), undifferentiated

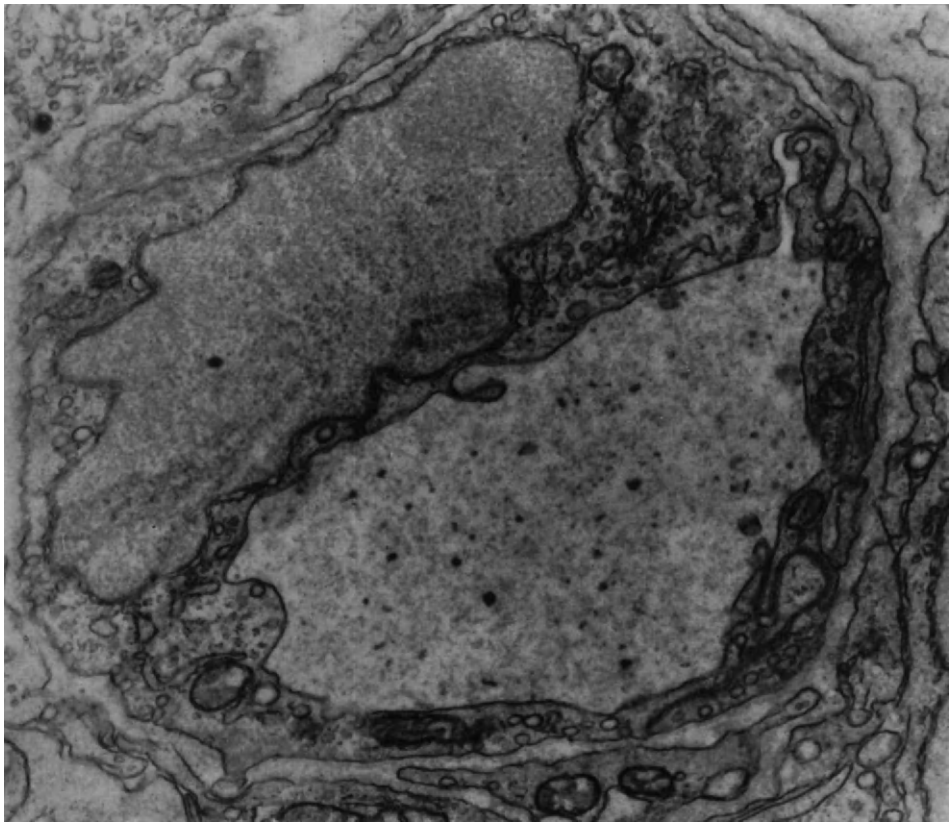

FIG. 13.12. An electron micrograph of a transverse section of a muscular type capillary. One endothelial cell nucleus is visible in the upper left hand part of the micrograph. Note also the pinocytotic vesicles in the cytoplasm.

0·5 μm.

amoeboid mesenchyme cells or reticular cells and fibres as a sheath: in addition there are usually adventitial cells such as fixed macrophages and fibroblasts, also undifferentiated mesenchymal cells, and a few cells belonging to accompanying nerve fibres.

It should be pointed out that the capillary endothelium does not directly touch the elements of other tissues: there is always an intervening layer, or basal lamina of connective tissue. The endothelial cells as a rule fit closely together; fluid from the blood reaches the tissues either by passing through the endothelial cells or between the cells, diffusing through the intercellular matrix. Amoeboid cells pass through the wall by actively pushing between the cells, or even occasionally by passing through them.

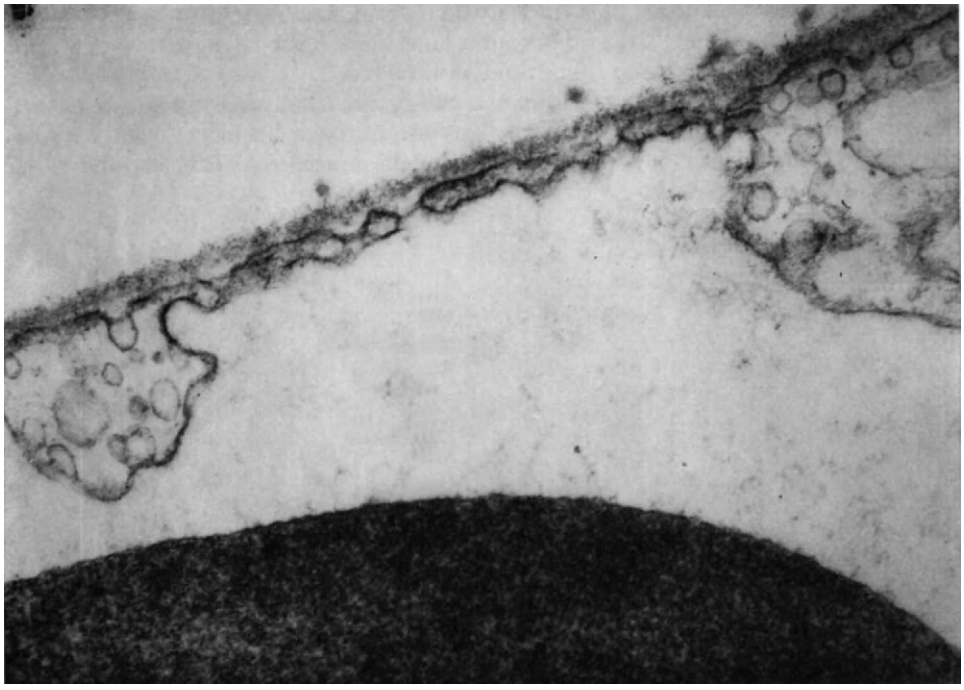

FIG. 13.13A. Electron micrograph of part of the endothelial wall of a fenestrated capillary. Note the basal lamina, the extreme thinness of the wall at the fenestrae and the red blood corpuscle at the bottom of the micrograph. 0·25 μm.

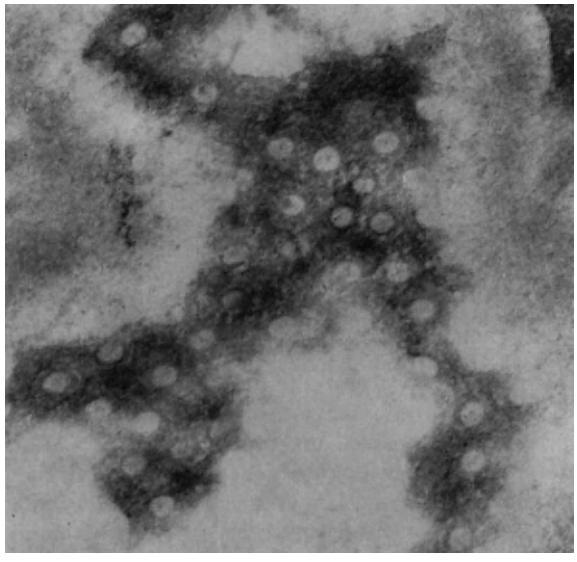

FIG. 13.13B. Part of the wall of a fenestrated capillary seen in tangential view. The circular fenestrae are clearly visible. 0·25 μm.

SINUSOIDS

Sinusoids are large blood spaces to be distinguished from capillaries in five respects.

(*a*) The cell wall of a capillary is complete and consists of an endothelium; that of a sinusoid is irregular, and consists of both endothelial cells and cells of the reticulo-endothelial system, some of which are phagocytic and in some locations (e.g. liver) it may be incomplete, with the basal lamina absent, so that the blood is in places in direct contact with the tissue cells (see Fig. 21.10).

(*b*) A capillary is always surrounded by a thin layer of connective tissue, whereas a sinusoid has only a network of reticular fibres and very often they may collapse when they are empty of blood.

(*c*) A capillary is always the connecting link between arterial and venous vessels, whereas a sinusoid may connect vessels of the same type, usually venous.

(*d*) A sinusoid usually has a relatively wide lumen (20 μm. or more) so that blood flow in it is very sluggish.

(*e*) A capillary develops in embryonic connective tissue and grows only by the addition of new vessel-forming cells at its ends. A sinusoid appears as a large space lined by endothelium and connected with the venous system: the elements of the developing organ push into this, the cells being thus in direct contact with the invaginated endothelium. The endothelium in large part disappears and is partly replaced by reticulo-endothelial cells, and the blood comes to be in direct contact with the cells of the organ.

Sinusoids are found in the liver, spleen, adrenals, parathyroid, pituitary, carotid and coccygeal bodies, and elsewhere.

The Blood Vessels of Blood Vessels (Vasa vasorum)

The walls of medium and large-sized arteries and veins possess blood vessels: these arise from adjacent small arteries and make a dense capillary network in the adventitia. In the aorta

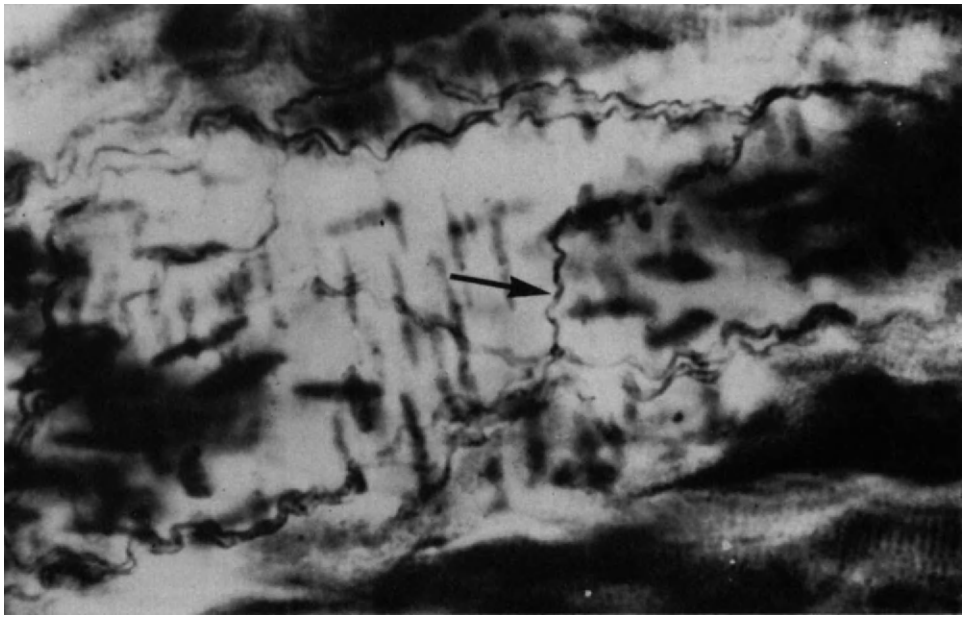

FIG. 13.14. Part of a vessel wall, stained to show some of the plexiform nerve fibres (arrow). 10 μm.

a few of these vasa vasorum may penetrate into all the coats and ultimately drain into the lumen of the vessel, but in general the vasa vasorum supply only the adventitia and the external part of the tunica media coat. The remainder of the wall of the blood vessel is nourished from within the vessel. (According to some, the tunica intima and the tunica media are supplied by a network of small vessels anastomosing with those of the adventitia.)

The Lymphatics of Blood Vessels

True lymphatic vessels are found in abundance in the adventitial and peri-adventitial tissue.

Interstitial lymph circulates freely in the intima and media coats, diffusing from the plasma in the lumen of the vessels and passing easily between the fenestrated elastic laminae. The disposition of these elements favours an easy flow in the direction of the long axis of the vessel; the direction of flow, as favoured by the blood pressure, is always from within outwards.

The Nerves of Blood Vessels (Figs. 13.14, 13.15)

All blood vessels are supplied with nerve fibres. These are both vasomotor and sensory in function. The vasomotor fibres, either constrictor or dilator, are non-myelinated autonomic fibres: they form a plexus in the adventitial coat and end as varicose threads on or in the smooth muscle cells of the media. Since the neurotransmitter substance at these nerve endings is adrenalin or a related compound, they may easily be visualized by the technique of fluorescence microscopy, after treatment of the tissue with formaldehyde vapour which makes the adrenalin fluoresce vividly. An example of this technique is illustrated in Fig. 13.15 which shows the innervation of the small blood vessels in the iris of the eye. Sensory endings are found in the adventitial coat and occasionally in the intima: these are supplied by myelinated

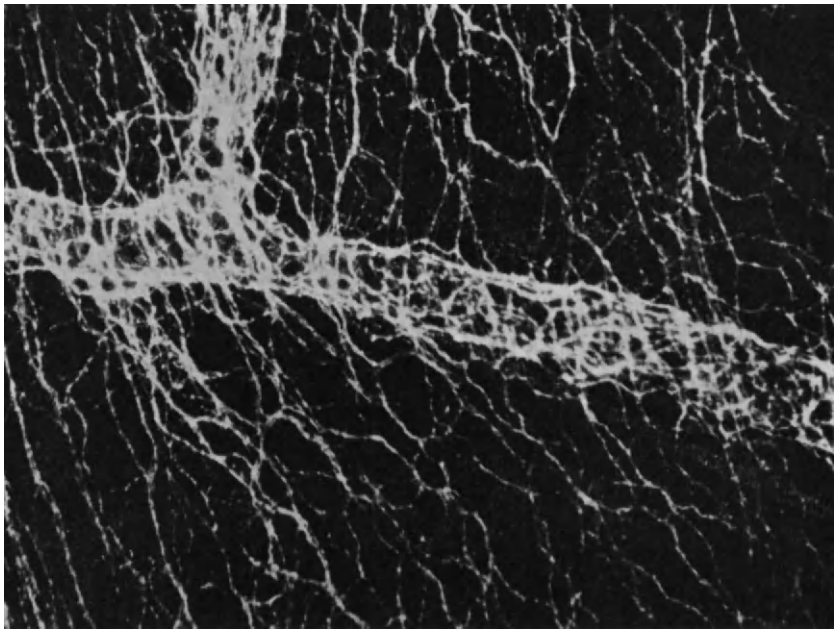

FIG. 13.15. A small blood vessel in the iris of a rat. The catecholamines in the terminals of the sympathetic vasomotor nerves appear bright due to their fluorescence following treatment with formaldehyde.

10 μm.

fibres. In some places, as in the arch of the aorta and in the carotid sinus vessels, the sensory endings are particularly well developed.

Even the capillaries are supplied by a rich non-myelinated nervous network.

CONNECTIONS BETWEEN ARTERIAL AND VENOUS SYSTEMS

(1) The Capillary Bed

This has already been described (p. 182).

(2) Arterio-venous Anastomoses

In many parts of the body, especially those where metabolic needs vary widely from time to time, arterioles are directly connected to venules by linking channels. These arterio-venous anastomoses, as they are called, have muscular walls which are richly supplied with vaso-motor sympathetic nerves. Constriction of the anastomoscs forces the blood to circulate through the capillary bed, whereas relaxation of the arterio-venous anastomosis allows the blood to bypass the capillaries entirely.

(3) The Glomus (Fig. 13.16)

This specialized kind of arterio-venous anastomosis is found typically in the skin at the cutaneous-subcutaneous junction, particularly in the pulp of the digits, in the nail bed, in the lips, in the tip of the tongue and in the nose. An arteriole, just before it gives rise to its capillary network, *also* gives rise to two to four short tortuous vessels: these are the anastomotic channels of the glomus, and their origin is marked by a cushion-like elevation of endothelium and muscle that acts like a valve. The thick wall of the canal has no elastic tissue, but contains a collagenous reticulum and short contractile cells whose contraction completely

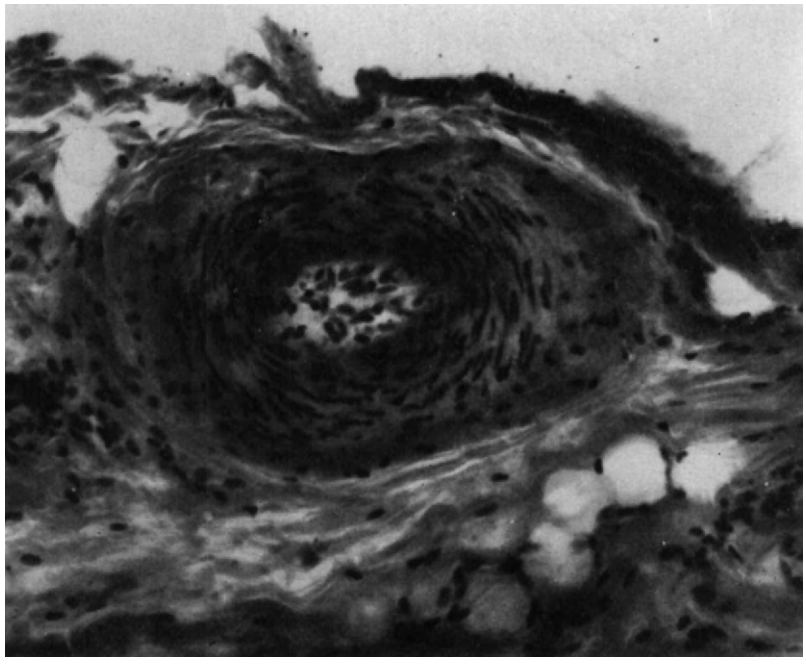

FIG. 13.16. Micrograph of part of a glomus in transverse section. Note the numerous nuclei of the contractile cells arranged in a circular fashion around the lumen. 50 μm.

occludes the lumen: therefore, when the canals are shut, all the blood must pass through the capillary network. These arterial canals open directly into large venous segments, whose walls consist of an endothelium resting on a fibro-elastic membrane and contain no muscle: these open into a vein. The whole tangle of arterial and venous vessels belonging to one afferent artery is enclosed in a connective tissue mass and is known as a *glomus*. The wall of the short-circuiting canal is very cellular: the cells are epithelioid and probably similar to the pericytes of the capillaries and are richly innervated with both sensory and motor fibres. The arterial segments contract and relax, the state of contraction varying with many conditions, particularly with alterations of temperature. The glomus is concerned with regulating the peripheral circulation, and hence the local temperature and tissue nutrition. It develops after birth and disappears in old age.

Certain vascular affections of the extremities are associated with abnormality in structure or functioning of the glomus mechanism. In most tissues of the body similar but smaller shunts occur between arteries and veins.

(4) Other Special Modifications

The most important of these are as follows:

(*a*) **Portal Systems.** Several places occur in the body where blood is collected from one set

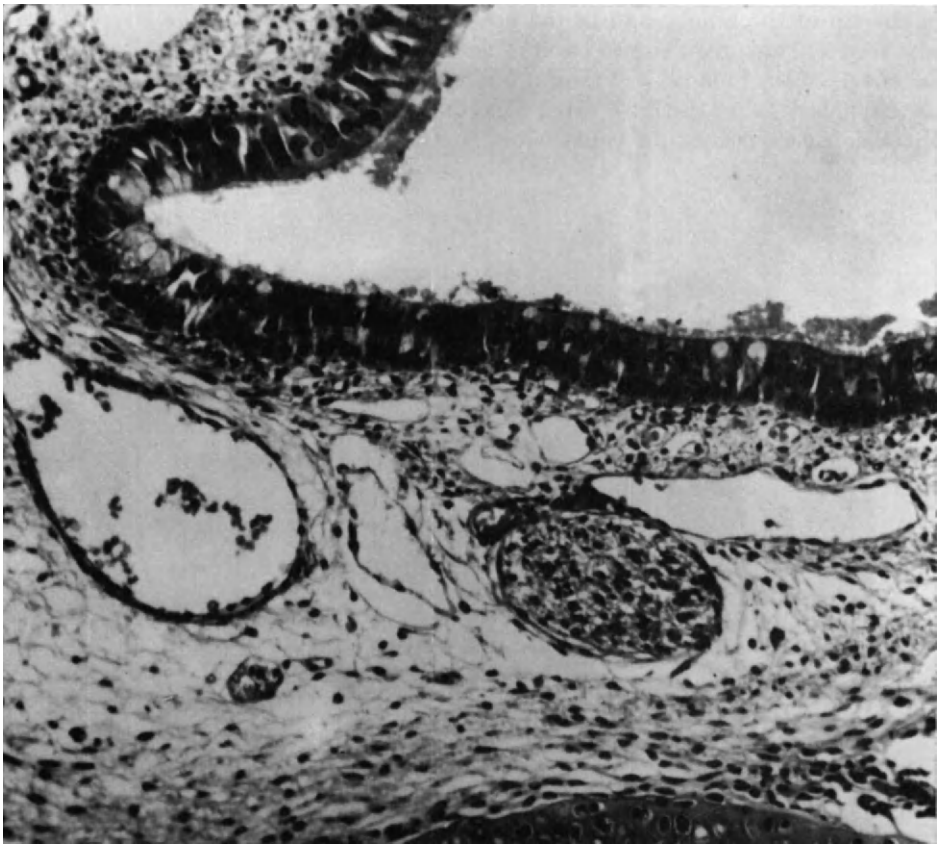

FIG. 13.17. A micrograph of a section through the ciliated nasal mucosa. There is a rich vascular plexus in the connective tissue below the epithelium; this serves to warm and humidify the air as it passes over the epithelium.

70 μm.

of capillaries and passes to a larger type of vessel or vessels before returning to the systemic circulation. This arrangement is called a *portal system*. An artery may also ramify into a number of capillaries which are then collected again into a larger vessel of the original type. This occurs in the arteries supplying the glomeruli of the kidney (see p. 345) whilst the portal vein itself is an example of the former arrangement of vessels. The portal vein is derived from a capillary network in the intestine; it breaks up into a sinusoidal network in the substance of the liver and the blood is ultimately collected again in the hepatic veins (see p. 321). A portal system is also involved in the blood supply of the anterior lobe of the pituitary gland (see p. 237).

(*b*) **Cavernous Erectile Tissue.** This special tissue derived from capillaries is considered in connection with the sex organs (see p. 366).

(*c*) **Placental Blood Spaces.** The maternal blood circulates in wide spaces that are lined by a syncitium of ectodermal origin instead of by an endothelium.

(*d*) **Venous Plexuses.** In the nose, on the lower conchae, are found rich venous plexuses just beneath the epithelium (Fig. 13.17). The walls are very thin and the cavities are relatively large. Their purpose is to warm and humidify the entering air.

DEVELOPMENT OF BLOOD VESSELS

Blood vessels are always developed in connective tissue, or, in the early stages, in the mesenchyme or primitive connective tissue. Blood vessels and heart all arise as simple tubes, i.e. their walls consist at first of one layer only of flat endothelial cells. Groups of branching mesenchymal cells in the embryonic body flatten themselves round a space containing fluid, and in this way the heart and main vessels are first laid down. They unite together and with the vessels of the area vasculosa of the yolk sac, and the circulation is established.

After the closed circulatory system has been established new vessels arise only by budding from pre-existing capillaries. During development the arrangement of the blood vessel network changes continually by fusing, widening or constricting, or even breaking and disappearing of individual vessels.

Vascular budding occurs during development, in inflammation, in repair, and in tumour growth: the process is always the same, the sprouts being at first solid and then becoming hollowed out, or canalized.

Elastic fibres are found in the blood vessel walls even before the sixteenth foetal week in organs where it is of vital importance that the vessels should not be accidentally occluded, such as the brain and the heart.

In the development of the heart and of the larger blood vessels the tube is at first laid down as an endothelium, and the wall is then thickened by the addition of new elements from the surrounding mesenchyme; these then differentiate to give the various fibres and cells that constitute the fully-developed wall.

REACTIONS OF BLOOD VESSELS TO VARIOUS CONDITIONS

(1) Variations with Age

The final differentiation of the various components of the blood vessel walls is not complete until adult life. The three main coats of the arteries are acquired during the fourth foetal month, but the intima of the aorta is not complete for about 30 years. The small arteries and the muscular arteries do not show such marked changes with age as do those of the elastic type. At birth there is no subendothelial tissue in the arteries, but with increasing age the formation of collagen and elastic fibres produces a progressive thickening. In childhood the intima of the aorta has a highly developed layer of circular elastic fibres.

The arteries are working continuously, so that it is not surprising that they show regressive

changes with increasing age. In the larger elastic vessels, particularly the aorta, there is often a patchy irregular thickening of the intima, with lipid deposition and degenerative changes. This condition is known as *atherosclerosis*. In the muscular arteries the elastic tissue in the tunica media becomes infiltrated with calcium salts, with a consequent loss of elasticity. This deposition of calcium salts occurs in the region where the part of the wall nourished from within adjoins the part nourished from without, and consequent disturbances of the lymph distribution further affect the calcium deposition.

(2) Effects of Mechanical Factors

(*a*) **Varicose Veins.** Certain veins are prone to dilatation from various causes. Such distension may cause partial incompetence of the valves, and where the action of gravity is marked (as in the legs) the weight of the column of blood still further increases the dilatation. In the region of varicosity the muscle and elastic tissues atrophy, leaving a wall of fibrous tissue only.

(*b*) **Alterations in Blood Flow.** The calibre of a vessel depends on the blood flow; dilatation follows an increase and constriction follows a decrease in flow. The length of the vessel depends chiefly on the growth of surrounding parts and the thickening of the wall depends on its expansion by the blood. The vessel retains the capacity of adjusting itself to alterations in these factors throughout life. Thus, after amputation, the cut vessels become obliterated, and following a blockage of a vessel a collateral circulation is established.

(*c*) **Aneurism.** A weakening of the media coat of an arterial wall from any cause is liable to produce a localized dilatation, or aneurism.

(3) Reactions to Nutritional Disturbances and to Toxic Processes

Any condition causing damage to the protective endothelium of blood vessels may result in a local clotting (thrombosis); it may also result in alteration of the osmotic exchanges and in an increased migration of leucocytes through the vessel wall (*diapedesis*).

New blood vessels may be formed from capillaries by proliferation of the endothelial cells which grow out at first in columns.

The intima and the media coats are particularly liable to be affected by the circulating blood because they are nourished directly from the plasma. The commonest degenerative change indicating a reaction to injurious conditions is the accumulation of fat, particularly in the intima layer. The disposition of the tissue elements of the intima and media coats, as explained above, favours the flow of the interstitial lymph derived from the plasma within the vessel in a longitudinal direction along the vessel wall rather than transversely through its thickness: this accounts for the direction of spread of many lesions of the walls. Any condition that interferes with the flow of lymph produces lesions in the media coat and usually in the intima also, because the lymph is their chief source of nourishment and the only path for removal of their metabolites.

THE HEART

The heart should be regarded as a locally enlarged part of the vascular system. It lies within the pericardial sac, a serous cavity whose visceral layer is reflected over the substance of the heart as the epicardium (or visceral layer of the pericardium). The wall of the heart (Fig. 13.18) consists of three layers:

(1) The internal layer or *endocardium*, in contact with the blood.
(2) The intermediate cardiac muscular tissue, or *myocardium*.
(3) The external covering layer, *the epicardium or visceral layer of the pericardium*.

Endocardium

The endocardium is a thin membrane, thicker in the atria than in the ventricles; it is lined with a flattened endothelium continuous with that of the intima of the blood vessels. This rests

on a thin, subendothelial layer of connective tissue of varying thickness containing collagen and elastic fibres, fibroblasts, and in parts some smooth muscle cells. Externally the endocardium is in most parts of the heart wall continued into a thick subendocardial layer of loose connective tissue: this contains blood vessels, nerves and branches of the conducting system (see p. 195), and becomes directly continuous with the interstitial tissue of the myocardium. The endocardium internal to the sub-endocardial layer contains no blood vessels, and is nourished by imbibition from the circulating blood as is the case for the inner coat of the blood vessels.

Rings of dense connective tissue are found surrounding the openings between the heart and the blood vessels, and also between the atria and the ventricles. These *annuli fibrosi*, together with dense connective tissue in the interventricular septum serve for attachment of the valves and of the muscle fibres and form what is known as the *cardiac skeleton*. In aged individuals this tissue may become calcified.

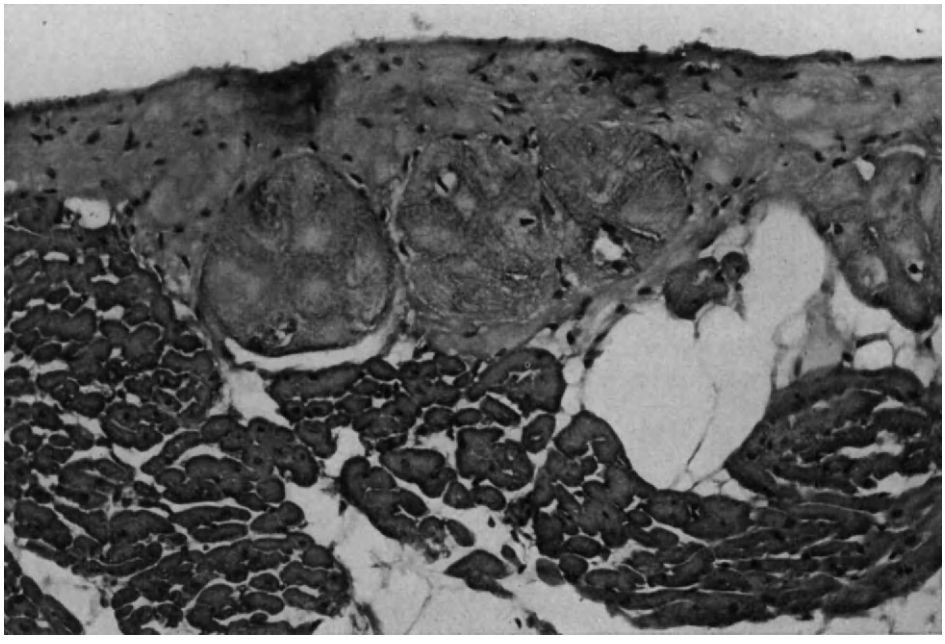

FIG. 13.18. A section of the heart wall. The endocardium is at the top of the picture with a number of large Purkinje fibres cut in transverse section just beneath it. 50 μm.

The heart *valves* consist of folds of endocardium greatly strengthened by a central plate of dense fibro-elastic connective tissue that is attached at the root of the valve to the annuli fibrosi. The valves are covered by endothelium (Fig. 13.19): in the semilunar valves this is directly continuous with that of the arteries and in these valves the side of the valve towards the artery is particularly strengthened with fibrous tissue in order to withstand the back rush of blood when they close. Macrophages are present in the subendothelial tissue. The vascular supply is confined to the base of the valve: the distal thin part of the cusp has normally no blood vessels and consequently shows little protective reaction. In the response to inflammation (p. 427) blood vessels grow out into the diseased valve from the vessels at the base. The valves possess no lymphatics. In the foetus they contain muscle.

Myocardium

The muscular mass of the heart tissue varies in thickness in different parts, being thickest in the left ventricle and thinnest in the atria. The minute structure of cardiac muscle has been

already described (p. 131). The muscle is arranged in layers of very complex pattern, and between the fibres is found areolar tissue with large numbers of capillaries.

Epicardium (Visceral pericardium)

The epicardium, being a serous membrane, is covered on its free surface with a layer of mesothelial cells which are constantly changing their shape as the heart contracts and relaxes.

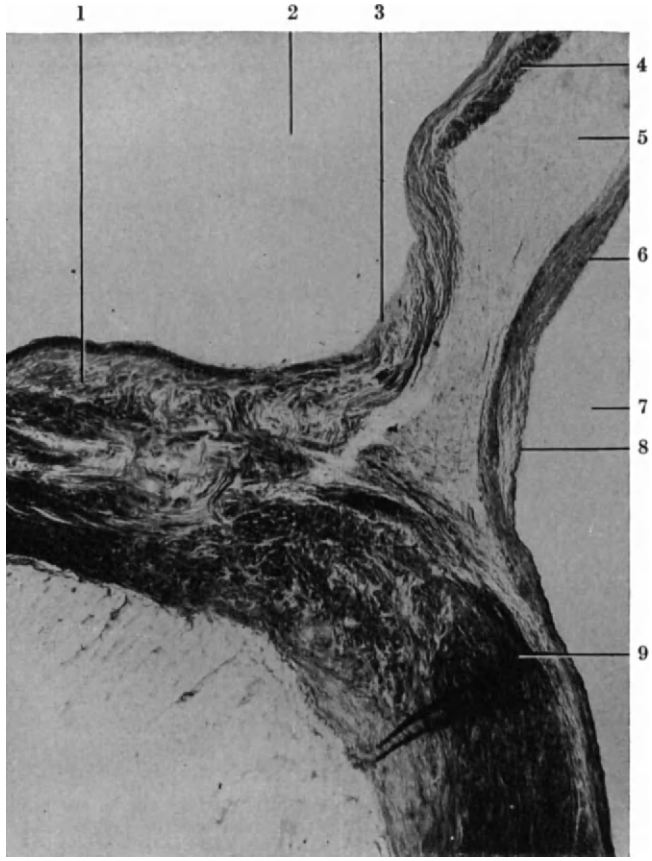

FIG. 13.19. A section through one flap of the aortic semi-lunar valve of a full term human foetus. 1, wall of aorta. 2, lumen of aorta. 3, endothelium of aorta prolonged over the valve. 4, fibrous thickening of valve on aortic side. 5, connective tissue of valve. 6, elastic fibres in valve on cardiac side. 7, cavity of ventricle of heart. 8, endothelium of ventricle over valve. 9, wall of ventricle.

Beneath the mesothelium is a layer of connective tissue containing many elastic fibres, particularly in the deeper part. Beneath, in the sub-epicardial layer, are found the main blood vessels, lymphatics and nerves supplying the heart: these are embedded in connective tissue that is continuous with that between the muscle bundles of the myocardium. Some fat is always present in the epicardial tissue, large variations in the amount present from time to time being quite normal.

The parietal layer of the epicardium is a serous membrane of the usual type (see p. 78).

The Impulse-Conducting System

All parts of the heart do not contract simultaneously: the motor impulse arises in the part developed from the embryonic sinus venosus, i.e. at the point of entry of the superior vena cava into the right atrium, and spreads first to the atria and then to the ventricles. The conduction of the impulse is carried out by the network of branching, anastomosing, atypical cardiac muscle fibres that constitute the Purkinje system. These fibres are collected into two masses, the *sinu-atrial node* and the *atrio-ventricular node*: in the embryo at least these nodes are connected by fibres of the same system, and in the fully developed condition the fibres are, in addition, continued as a bundle, the *atrio-ventricular bundle* (bundle of His), which passes to the inter-ventricular septum and then splits into branches that ultimately supply both

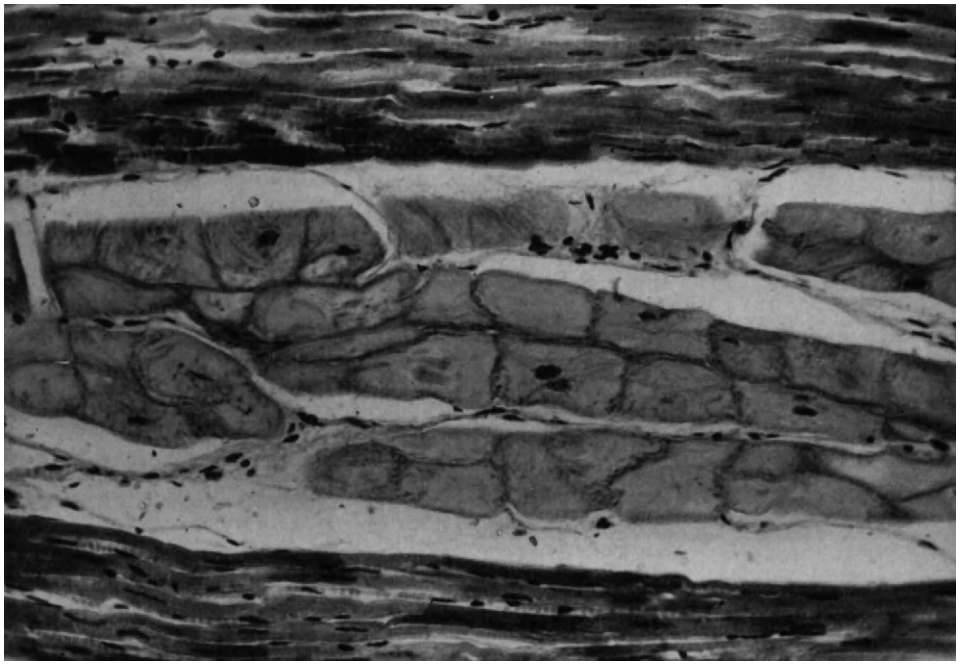

FIG. 13.20. Purkinje tissue of heart. Note the large size of the Purkinje fibres as compared with typical cardiac muscle cells (seen here in L.S.) at the upper and lower edges of the micrograph. 40 μm.

ventricles. If the atrio-ventricular bundle is damaged the normal rhythm of the heart beat is lost. (For a description of the exact distribution of the Purkinje tissue the student is referred to detailed accounts of the topography of the heart.)

The typical Purkinje fibres are large, long cells, that are differentiated only at their periphery into fibrils and cross-striations, and contain one or more nuclei in the central, clear zone (Fig. 13.20): the cells are rich in glycogen. In man these fibres are not always very clearly marked off from ordinary cardiac muscle fibres. The atrio-ventricular bundle contains very few typical Purkinje cells, and in the sinu-atrial node the cells are almost spindle-shaped and branched, with cross-striations, and are difficult to distinguish from the rest of the myocardium. In the atrio-ventricular node the fibres are short and thick, with branches and anastomoses. All the fibres of this system are transversely striated, and contain one or more central nuclei in a clear perinuclear zone.

The whole ramifying system of conduction fibres is covered by a thin connective tissue sheath which also encloses nerve fibres and small blood vessels and separates it from the rest of the myocardium: in man the connective tissue sheath is in most places too thin to be easily recognizable.

Blood Vessels

The coronary arteries, arising in the aortic sinus, supply the whole of the myocardium: the collecting coronary veins empty either directly into the right atrium or by way of the coronary sinus. The endocardium proper contains no blood vessels, those of the sub-endocardial layer belonging actually to the myocardium. The attached parts of the valves have a rich, fine capillary plexus. The whole of the conducting system, particularly the nodes, is richly supplied with blood vessels.

Lymphatics

The blood vessels of the myocardium are accompanied by small lymphatic vessels. Epicardium, endocardium and valves are all supplied with lymphatic capillaries, the rich network of sub-epicardial and sub-endocardial lymph vessels being in continuity with that of the myocardium.

Nerves

The heart is supplied with numerous nerve fibres of sympathetic and of parasympathetic vagal origin. These form plexuses of mixed myelinated and non-myelinated fibres in each layer of the heart wall. Many small ganglia are present on the course of these networks, particularly in the sub-epicardial tissue and in the region of the nodes: the atrio-ventricular

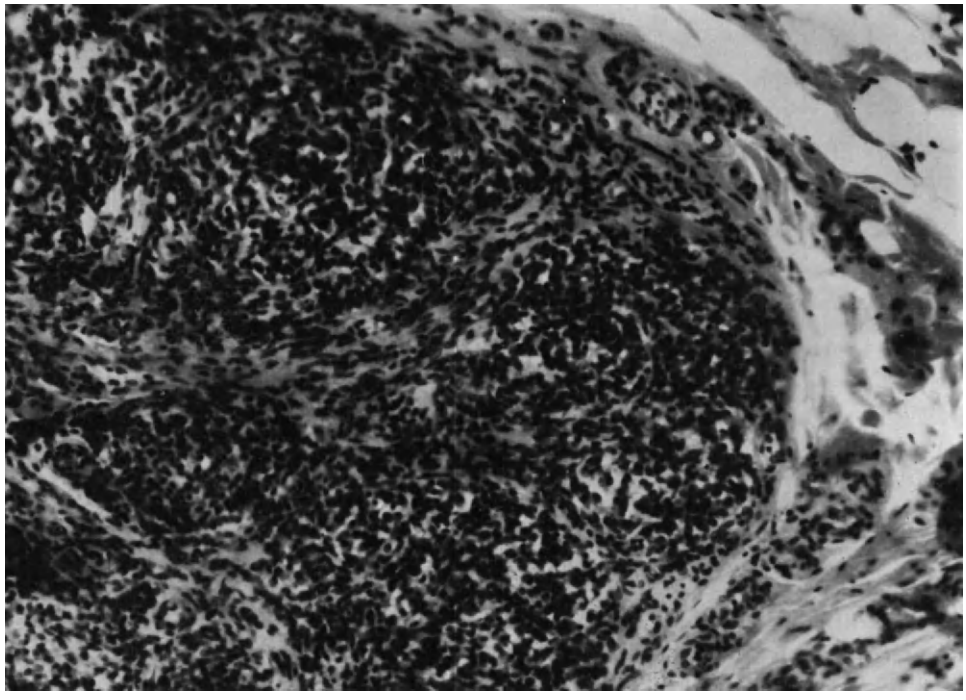

FIG. 13.21. A section of part of the wall of the carotid body. The nuclei are those of the epithelioid glomus cells.

bundle appears to be devoid of ganglion cells and but poorly provided with nerve fibres. Both endocardium and epicardium contain afferent nerve endings in the form of diffuse ramifications.

The Carotid and Aortic Bodies

The carotid bodies (Fig. 13.21) are small flattened bodies lying one on each side at the bifurcation of the common carotid artery. They consist of a dense fibrous framework supporting clumps of large polygonal epithelioid glomus cells. Each glomus is supplied by an arteriole and venule, the capillaries being sinusoidal in structure. There are many sympathetic nerve fibres and a very abundant sensory nerve supply from the ninth cranial nerve: the nerve endings lie in very close contact with the cells, and also with the lining of the sinuses. The relative amount of connective tissue increases with age. The organ is concerned with the afferent impulses underlying the reflex control of blood pressure and respiration: the nerve endings are sensitive to chemical stimuli indicating a fall in the pH of the blood, a decrease in its oxygen content or an increase in the carbon dioxide concentration. The impulses are carried by the glossopharyngeal nerve.

The aortic body is similar in structure and function to the carotid bodies. It is usually found applied to the arch of the aorta. The impulses are carried by the depressor fibres of the vagus nerve.

Coccygeal body. This is a small median organ lying in front of the apex of the coccyx. It has the same general structure as the carotid body.

Reactions of the Heart to Various Conditions

(1) Variations with Age and Activity

Physiological hypertrophy of the myocardial tissue results from hard and prolonged physical work: a small degree of enlargement also occurs in pregnancy.

The adipose tissue of the epicardium varies greatly in amount: in obesity the fat cells multiply and may spread along the blood vessels to infiltrate between the myocardial fibres, and may even interfere with the work of the heart. In emaciation the fat cells atrophy and tend to disappear.

In old age the heart shows some degree of senile atrophy: the myocardial fibres remain of the same length, but shrink in bulk, and excess of yellow pigment appears at the poles of the nuclei.

(2) Reactions in Pathological Conditions

The specialized reactions do not fall within the scope of this book, but an understanding of such changes will be assisted if the student remembers that all the parts of the heart are dependent upon one another, and that a lesion of one may affect the others. For example, the endocardium is nourished by imbibition from the circulating blood, and is consequently readily affected by blood toxins: in this way the valves may be injured with resulting interference with the normal filling and emptying of the heart cavities, and this in turn will be followed by compensatory changes of the myocardial tissue. Again, lesions of the coronary vessels must be attended by profound changes in the contractile and conducting tissues.

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CHAPTER 14

THE LYMPHATIC SYSTEM, THYMUS AND SPLEEN

THE LYMPHATIC SYSTEM

The lymphatic system consists of a closed series of vessels and lymphoid organs. The lymph vessels, or lymphatics as they are usually known, form a closed series of channels which begin blindly in the tissues. They are lined with an endothelium and eventually lead into larger lymph ducts which in turn pass the lymph back into the vascular system in the angle between the subclavian vein and the internal jugular vein on each side of the body. Lymph itself is largely derived by transudation from the blood capillaries and contains the same proteins as blood plasma, though in smaller amounts. There is also a higher proportion of albumin to globulin, but the proportion of fibrinogen is lower than that of plasma. After a meal containing much fat, the lymph which drains from the small intestine contains small fat globules (chylomicrons) which may be so numerous as to render the appearance of the lymph turbid. The number of cells in the lymph varies greatly, but they are predominantly small lymphocytes.

The lymphoid organs are scattered along the course of the lymphatics; they filter the lymph as it flows through them and they also add lymphocytes to the lymph. The lymphoid tissue includes the *lymph nodes*, formerly often called "glands", isolated nodules of lymphoid tissue which may occur in almost any site in the body and the specialized lymphoid organs —the *thymus* and the *spleen*.

No lymphatics have been demonstrated in uncalcified cartilage, in epithelia, in the lens and sclera of the eye, in the internal ear, in the foetal part of the placenta, in the bone marrow, in the spleen pulp, or in the central nervous system.

Lymphatic Vessels

Lymphatic Capillaries

The lymph capillaries are found beginning in almost all parts of the body: they are surrounded by connective tissue and begin as a network usually close to the network of blood capillaries. The capillaries branch and anastomose, often having dilatations and blindly-ending projections (e.g. lacteals of intestinal villi) (Fig. 14.1). In histological preparations they are difficult to see, because the walls are thin and although extremely distensible they tend to collapse, and the lumen as a rule contains no formed elements to prevent its obliteration. During the absorption of fat from the small intestine the lymphatics become filled with the milky fluid and are then easily seen in the mesentery as the lacteals. It is also possible to make the course of the lymphatics visible by the retrograde injection of a soluble dye (Plate 2A). The wall of a lymphatic consists of a thin layer of a highly permeable endothelium, the cells of which show the sinuous cell outlines that was noted in the case of the blood capillaries. As the basal lamina is poorly developed and virtually indistinguishable, there is effectively nothing between the endothelium of a lymphatic and the surrounding connective tissue. In this respect there is a marked difference between the lymphatics and the capillaries which always appear to have a clearly marked basal lamina and are surrounded by pericytes.

Larger Lymphatic Vessels

The capillary networks drain into small lymphatic vessels. The walls of these contain a few longitudinally arranged collagen and elastic fibres and a few tangential smooth muscle cells. The larger vessels have thicker walls in which the typical three layers can sometimes be distinguished. The *intima* consists of endothelium and a thin layer of longitudinal elastic fibres. The *media* contains smooth muscle fibres for the most part arranged circularly, and some fine elastic fibres. The *adventitia* is the thickest coat and is composed of longitudinal and tangential collagen and elastic fibres and smooth muscle bundles. The adventitia is continued into the surrounding tissue. The walls of lymphatic vessels contain relatively little muscle and much elastic tissue.

As the vessels become larger the walls become thicker: they accompany the blood vessels and frequently make an anastomosing lymphatic network surrounding them. Lymphatics are

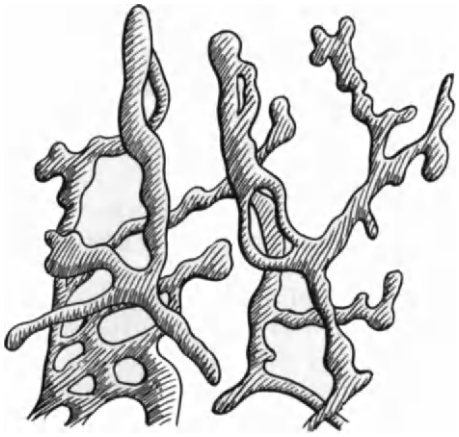


FIG. 14.1. A diagram of the appearance of a network of lymphatic capillaries in the superficial tissues of the ear. The lymph vessels have been filled by a retrograde injection of the dye Berlin blue. Note the blind endings and anastomoses between channels.

often situated near main arteries whose pulsation propagates the lymph in the direction determined by the valves which occur in lymphatics, just as in veins.

Thoracic Duct

All the lymphatics finally drain into either the right lymphatic duct or (on the left side of the body) the thoracic duct, the latter being the larger. Both ducts empty into the great veins at the root of the neck, the opening being guarded by valves. The wall of the thoracic duct is characterized by a thick tunica media consisting of muscle bundles that are chiefly longitudinal and separated by abundant connective tissue (see Fig. 14.2). In different regions it varies greatly in both size of lumen and thickness of wall.

Valves

Valves occur in pairs, being placed opposite to one another, with their free edges directed with the flow of fluid. As in the veins, the valves are folds of intima, with a little connective tissue at the base. Above the valves the lymphatic vessel is expanded and the muscle in the middle coat of the wall is particularly well developed. The valves are found in small lymphatics as well as in large vessels.

Blood Vessels of the Lymphatics

It is usual to find a small artery and a small vein and a lymphatic vessel running parallel with one another: capillaries between the blood vessels make a network on the surface of the lymphatic. The large lymphatics are provided with small blood vessels supplying their outer coat and extending into the media, as is the case with the large blood vessels.

Nerves of the Lymphatics

The lymphatic vessels are richly supplied with non-myelinated nerve fibres: these form networks in the adventitia and media and sometimes extend as a sub-endothelial plexus. Both sensory and motor nerve endings are present.

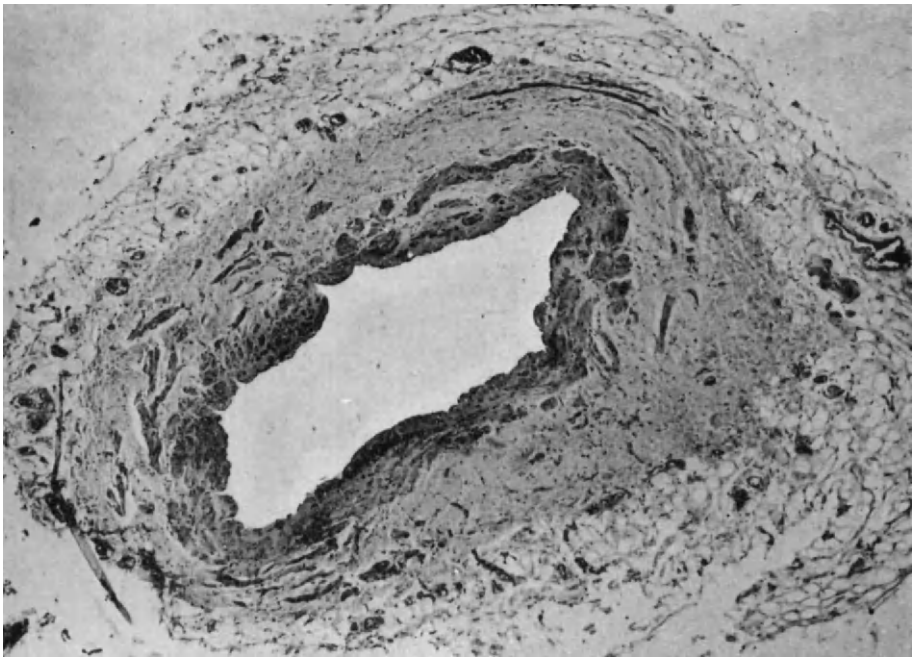


FIG. 14.2. Photomicrograph of a transverse section of the human thoracic duct. The longitudinal muscle bundles in the tunica media may be clearly seen. 0·5 mm.

Lymph Nodes

Lymphoid tissue is distributed very widely throughout the body, and the lymph nodes are only special collections of this tissue: they are scattered about the body in large numbers, often lying in groups. Detailed information on their distribution may be obtained from a text-book on gross anatomy. They are rounded structures lying on the course of lymphatic vessels, so that the stream of lymph filters through the lymph node. There is usually a slight depression at one side, the *hilum*: here the blood vessels enter and leave and the lymphatic vessels leave the node: the lymphatic vessels may enter the node at many places. The lymph nodes serve to filter the lymph and, at the same time, allow lymphocytes and other cells, together with gamma globulins (antibodies) to be added to the lymph and eventually to the blood stream.

A typical lymph node (Fig. 14.3) varies from 1–25 mm. in diameter; it is surrounded by a dense connective tissue *capsule* that may contain smooth muscle fibres: this sends fibrous *trabeculae* (or septa) into the substance of the node. The fibres of the trabeculae are continuous

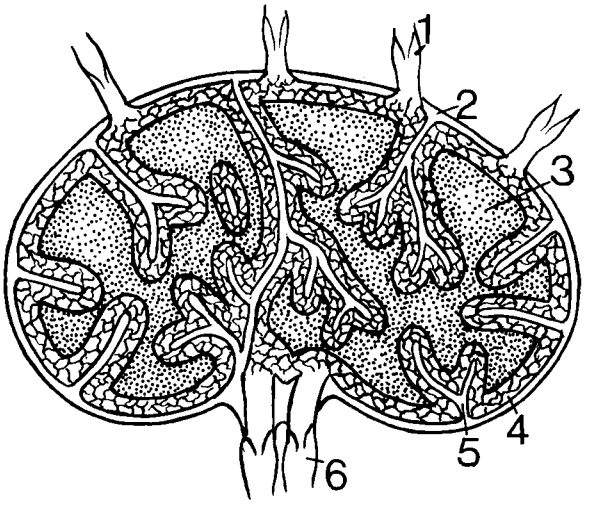


FIG. 14.3. A diagram illustrating the structural features of a generalized lymph node. 1, afferent lymphatic. 2, capsule. 3, cortex. 4, sub-capsular sinus. 5, trabecula. 6, efferent lymphatic.

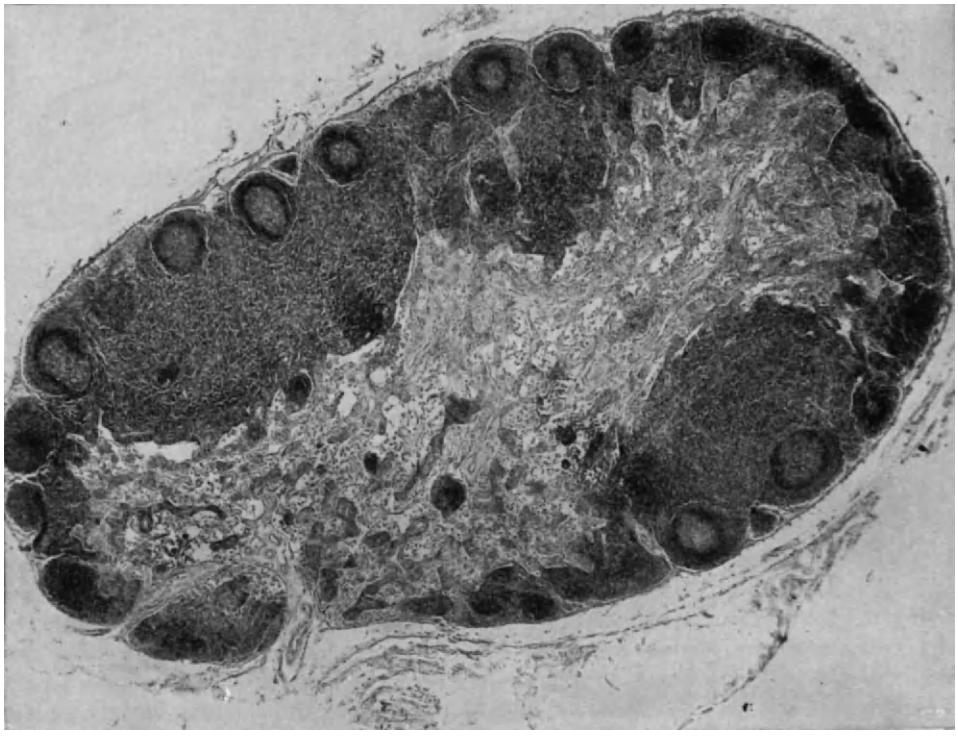


FIG. 14.4. A photomicrograph of a section of a human lymph node. Note the arrangement of cortical and medullary tissue, the hilum of the node and the cortical lymphoid nodules with their lightly staining germinal centres.

500 μm.

with the *reticular fibres* that make a framework to support the free cells. The substance of the node is often arranged as a dense *cortex* and a less densely-packed *medulla* (Figs. 14.4, 14.6); in the cortex are found the *lymph nodules* or follicles. The cells in the meshes of the stroma are chiefly lymphocytes, but there are, in addition, many free phagocytic macrophages derived from the cellular reticulum. Some of these reticular cells flatten out to line the lymph channels. Plasma cells are also frequently present.

The afferent lymphatic vessels pass into the sub-capsular (or cortical) lymphatic sinus which surrounds the organ immediately beneath the capsule (Fig. 14.6). This sinus is bridged over by fine branching fibres derived from the capsule and trabeculae, and by the highly

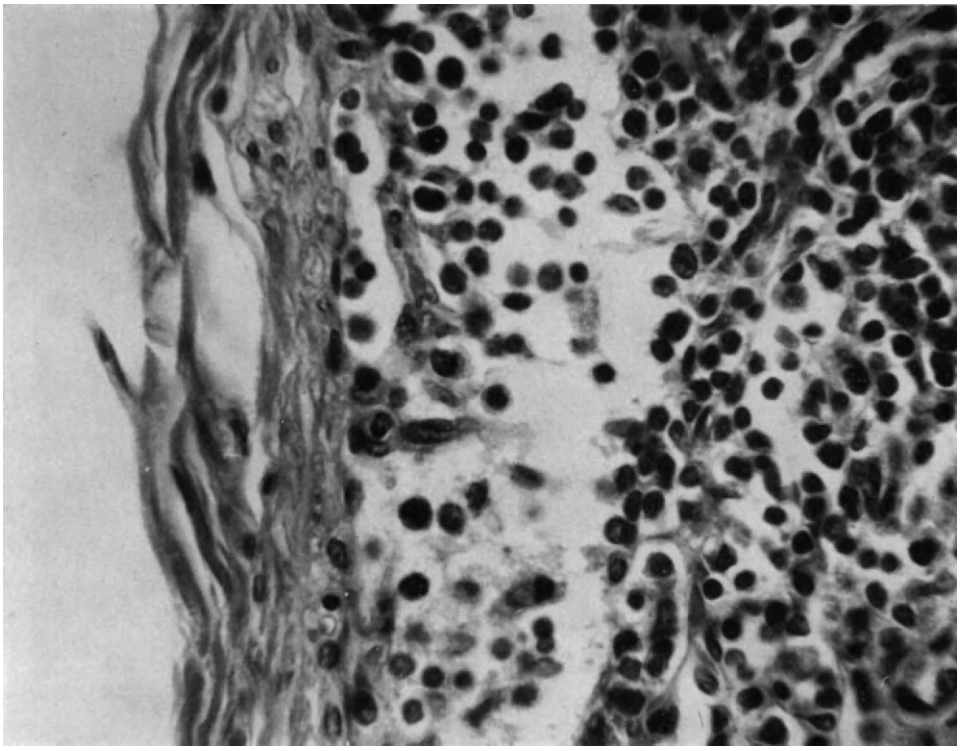


FIG. 14.5. A micrograph of the sub-capsular sinus of a lymph node. The fibrous tissue of the capsule is on the left and cortical tissue of the node on the right. The cells in the sinus are mainly lymphocytes. 30 μm.

phagocytic reticular cells (Plate 2B). The lymphoid tissue is everywhere separated from the capsule and trabeculae by such a lymphatic sinus bridged across by reticular fibres and cells. These sinuses anastomose throughout the organ and ultimately unite to form the efferent lymph channels.

The *primary lymph nodules* appear in the cortex of the gland as rounded masses of densely packed lymphocytes. The primary nodules may contain *germinal centres* (*secondary nodules*), and in this case a stained preparation shows the centre of the nodule light and the surrounding part dark. In the centre the cells are large, not closely packed, and some are undergoing mitosis: this is the germinal centre where lymphoblasts are giving rise to large lymphocytes. As a result of the cell division the surrounding cells are pushed outwards so that the peripheral zone contains densely packed small lymphocytes and consequently stains darkly.

Recent studies with tritium-labelled lymphocytes have shown that there is a circulation of lymphocytes: they are not only released into the circulation, but they also re-enter the lymphatic tissues from the blood stream by penetrating the walls of small blood vessels (post-capillary venules) in the lymph nodes.

Blood Vessels of Lymph Nodes

The arteries enter at the hilum and are distributed with the trabeculae, passing into the substance of the organ where they form capillary networks: the emerging veins also leave at

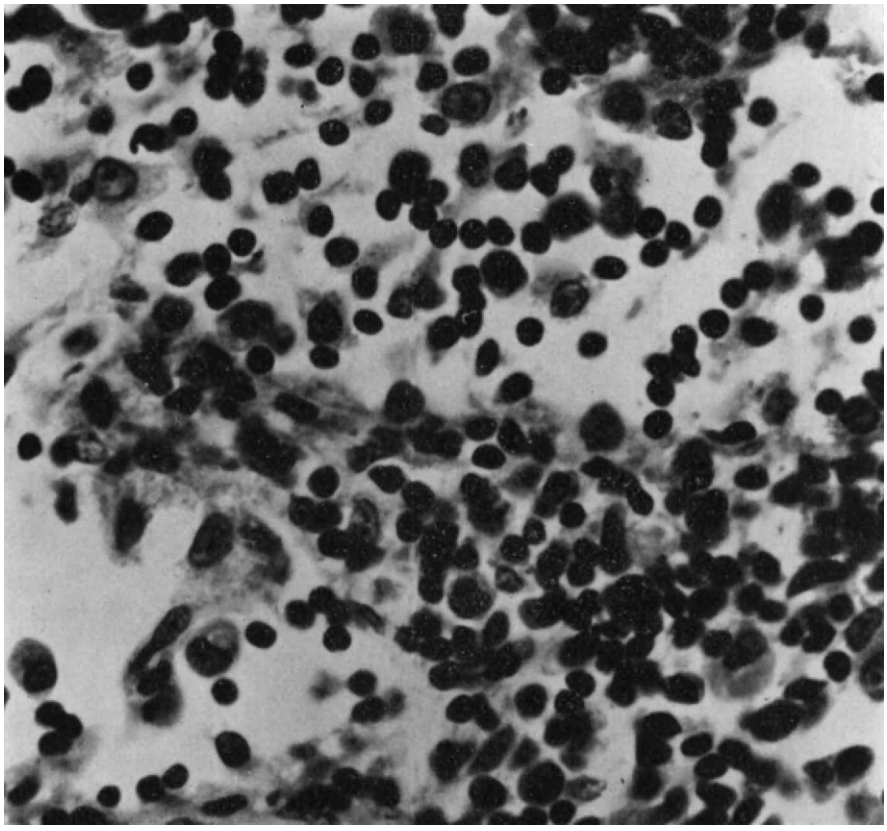


FIG. 14.6. Medullary tissue of a lymph node. Note the less closely packed texture of the medulla as compared to the cortex. Several plasma cells are visible (e.g. two in the bottom left hand corner of the micrograph) in the sinusoids. 20 μm.

the hilum. The post-capillary venules are distinguished by a high endothelium of cuboidal cells and a broad subendothelial layer of connective tissue. The E/M has shown that the endothelium of these vessels is often infiltrated with lymphocytes and this is the chief site of the re-entry of these cells into the lymph node. The lymphocytes appear to pass through the actual endothelial cell itself and not through the interstitial tissue in between the cells.

Nerves

The nerves enter with the blood vessels, and accompany them, making peri-vascular networks of non-myelinated fibres. They probably supply the smooth muscle of blood vessels.

Variations in Structure of Lymph Nodes

Lymph nodes may vary greatly in detailed structure, but their general plan is always the same.

The simplest node is an expansion on the course of a lymphatic vessel whose wall is thickened to form the capsule. The interior is occupied by the lymphoid tissue, i.e. a reticular framework of fibres and reticular cells permeated everywhere by lymph and lymphocytes: there may be a sub-capsular sinus and a central lymphoid nodule.

The more complex nodes have the same essential structure, but more sinuses and more lymphoid nodules. The lymph always enters the sub-capsular sinuses and passes from cortex to medulla, filtering through the nodules and ultimately leaving by the efferent lymph channels.

The trabeculae are best developed in large and peripheral lymph nodes, but are never very prominent in man: often the trabecular system is so slightly differentiated that the whole node appears as a diffuse mass of lymphoid tissue, with looser channels for the flow of lymph. This is the case in the lymph nodes of the alimentary canal and respiratory organs.

The relative amounts of cortical and medullary tissue vary greatly. The cortical tissue may completely surround the medulla or it may collect at a different pole of the node. Sometimes the medullary tissue may be immediately adjacent to the sub-capsular sinus for a large distance.

The special lymphoid structures include the tonsil (see p. 285), Peyer's patches in the intestine and the thymus (see p. 206).

Haemolymph Nodes

In the human foetus, and to a lesser extent in the human after birth, there are present small nodes similar in general structure to ordinary lymph nodes, but differing from them in that all the sinuses contain blood instead of lymph. They possess no lymphatics, and can be regarded as filters consisting of lymphoid tissue situated on the course of blood vessels. In man their existence is not certain, although some workers think that they may be found in the retro-peritoneal tissue and in the mediastinum. The spleen may, perhaps, be regarded as a modified haemolymph node (see p. 210).

Function of Lymph Nodes

Three main functions may be ascribed to the lymph nodes:

(1) Because of their large content of reticulo-endothelial cells, the lymph nodes constitute ideal filtration beds for the lymph which flows through them, so removing foreign bodies such as bacteria and any inorganic particulate matter.

(2) The lymphoid tissue itself, on the other hand, is specialized for the production of antibodies to any antigenic proteins which are found to occur in the lymph draining from any given region of the body.

(3) The lymph node is also important as a site of formation of the lymphocytes which enter the lymphatics and circulating blood.

Variations under Different Conditions

(*a*) Age and Activity

The germinal centres, involved more particularly than the rest of the lymph node in the active formation of lymphocytes, are not always present. They are not found in embryonic nor in senile tissues, and throughout post-natal life they may undergo a cyclic appearance and disappearance; they are largest and most active in the young. When completely inactive the actual germinal centre is difficult to make out. When activity begins a clear central area appears, increasing by proliferation of the cells and by a pushing of the mature lymphocytes to the periphery, so that the more darkly staining zone of closely packed lymphocytes once again

clearly delimits the germinal nodule from the surrounding tissue. Active lymph nodes tend to increase in volume (perhaps up to five times) and enlargement of the lymph nodes draining a region is indicative of inflammation.

(*b*) Pathological Processes

In order to understand the reactions of lymph nodes to pathological processes it is important to remember that the nodes always consist of two parts, namely, the lymphoid tissue (with its germinal nodules if present), and the lymph channels with the reticular system of cells and fibres. The lymphoid tissue proper is concerned primarily with the formation of lymphocytes, and under certain conditions granular leucocytes may also be found here. The lymphocytes are believed to exert a protective function against foreign proteins (i.e. antigens) by undergoing changes which transform them into the so-called plasma cells which are able to secrete antibodies. The reticular cells, on the other hand, are actively phagocytic, and take up particles brought to the node in the lymph stream; this accounts for the carbon granules commonly found in the histiocytic cells of the lymph nodes in the wall of the throat and bronchial tubes. Both, or only one, of the two structures present in the lymph node may respond to the pathological process; hyperplasia in response to irritation is common.

THE THYMUS

The thymus is an organ located in the superior mediastinum that undergoes great physiological variation in both size and structure, depending on age and on general conditions. It is largest relative to body weight in the foetus and in childhood up to the age of puberty, after which it undergoes gradual and continuous involution; this process may be greatly accelerated in the course of many infections and in wasting diseases (*accidental involution*) (see also p. 210).

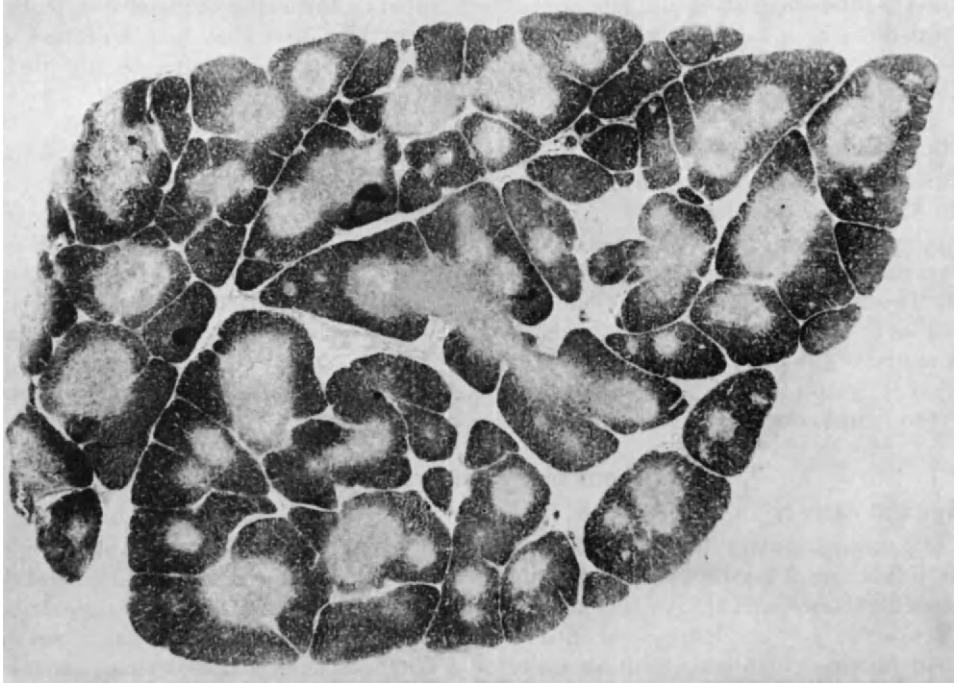


FIG. 14.7. A low-power micrograph of the thymus to show the prominent lobulation and the clear cut division of each lobule into cortex and medulla. 500 μm.

The organ has a thin connective tissue capsule, and consists of two main lobes each subdivided into innumerable lobules (Fig. 14.7), which are separated from one another by thin connective tissue septa that pass in from the capsule and carry the blood vessels. In section each lobule is seen to consist of a dense, darkly-staining peripheral cortex and of a looser, lightly-staining, central medulla. Actually the lobules are not separate; by following out serial sections it can be seen that the medullary tissue constitutes a central strand with projections at intervals, the projections being surrounded by cortical substance.

Cortex

The cortex looks very similar to the lymphoid tissue of the ordinary lymphatic nodes, but there are no primary or secondary follicles. It consists of epithelial cells which have become forced apart by the infiltration of densely packed masses of cells that morphologically look like lymphocytes; some writers term these cells *thymocytes* because they believe that they have an epithelial origin. The lymphoid cells of the cortex exhibit intense mitotic activity. The epithelial cells remain held together by desmosomes and so, in consequence of the lymphoid infiltration, they assume a branched, stellate appearance and are known as *epithelio-reticular cells*. There are no reticular fibres present, however, a feature in which the thymus differs from spleen and lymph nodes.

Medulla

The medulla consists largely of epithelio-reticular cells like those in the cortex and continuous with them: they are easily seen because the lymphocytes are much less numerous here than in the cortex. In addition, the medulla contains the characteristic *Hassall's corpuscles* (Figs. 14.8, 14.9). These bodies consist of concentrically arranged cells which stain with acid dyes; the innermost cells show signs of degeneration and hyalinization. The central cells may degenerate completely, and cysts or calcareous deposits appear. A paper by Blau (*Nature*, 1967, **215**, 1073) suggests that the Hassall's corpuscles may be actively phagocytic structures which become sites of aggregation of γ globulins and antigens. The intense thymic lymphopoesis has posed something of a problem; recent work has suggested that the migration of lymphocytes out of the thymus is not significant and that about 99 per cent of the lymphocytes produced in the thymus die there within four days. The large scale cell production has been postulated by some workers to generate (by random genetic variation) those lymphocytes with different immunological competences to cope with all possible invasive antigens and that the equally large scale lymphocyte destruction represents the destruction of those cells which bear antigenic patterns which would allow them to react against the body's *own* proteins. The thymic lymphocytes which are released from the thymus are thought to be long-lived (perhaps many years in the human) and constitute a large part of the bodies population of recirculating small lymphocytes. They have been shown to bear a specific theta antigen on their cell surfaces and hence are generally called "T cells"; it is probable that they are responsible for delayed hypersensitive reactions such as the rejection of skin grafts.

Thymectomy certainly causes atrophy of the other lymphoid tissue in the body and a lymphopaenia in the circulating blood; these may be corrected by thymus grafts so that it is concluded that the thymus is essential for the normal process of lymphopoesis in the body. A corollary of this is of course that the thymus is therefore essential for the adequate developing of a normal immunological response to foreign antigens. It is suggested that the thymus secretes a humoral factor which allows the competent lymphoid cells in the peripheral lymph nodes and other sites to proliferate after an antigenic challenge and so form the clones of lymphocytes and plasma cells which produce the necessary antibodies.

Much research is in progress at the present time on this and related problems; the interested reader is referred to papers by Miller & Osoba and Raff and to the books by D. Metcalf and L. Weiss for a summary of recent work and for extensive bibliographics.

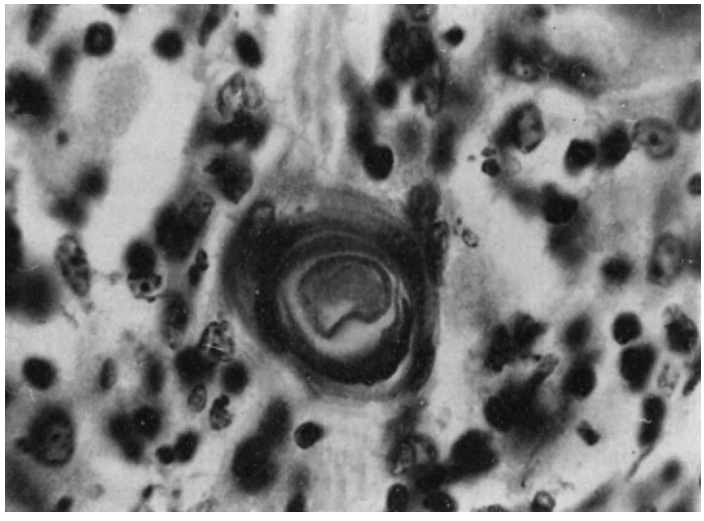


FIG. 14.8. A photomicrograph of a Hassall's corpuscle in the thymic medullary tissue. 20 μm.

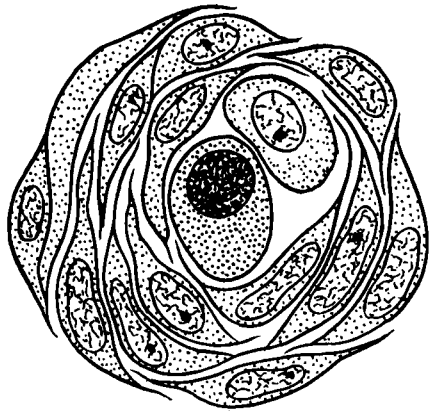


FIG. 14.9. A diagram of the organization of a Hassall's corpuscle. Note the concentrically arranged cells surrounding two central cells, one shown with a dark nucleus indicative of pyknosis and degeneration.

Blood Vessels and Lymphatics

The arteries penetrate into the organ to the junction between cortex and medulla: the main bulk of capillaries radiate out into the cortex, while some pass in to the medulla. The capillaries in the cortex are separated from the lymphocyte-containing stroma by a pericapillary space and a continuous or semicontinuous zone of epithelial cells. The veins leave both from the surface and from the medulla.

No lymph sinuses are present, but large issuing lymphatic vessels are found in the interlobular connective tissue.

Nerves

A few nerve fibres supply the organ and are derived from the vagus and from the sympathetic: most of them are probably vasomotor in function.

Development

The thymus arises as an outgrowth from the third pharyngeal (branchial) pouch on each side together with a contribution from the fourth pouch. At first there is a narrow lumen and a thick wall of cylindrical epithelium. The epithelium proliferates irregularly, the lumen is obliterated, and anastomosing strands spread into the surrounding mesenchyme. Transformation of the epithelial cells into epithelio-reticular cells occurs, and the small cells looking like lymphocytes appear. Some regard these cells as derived from the epithelial cells, but most workers believe that they are lymphocytes that have wandered in from the mesenchyme. Medulla and cortex are differentiated by peripheral aggregation of the lymphocytes, lobules are formed and separated by strands of connective tissue. Hassall's corpuscles are formed from hypertrophied and degenerating epithelio-reticular cells.

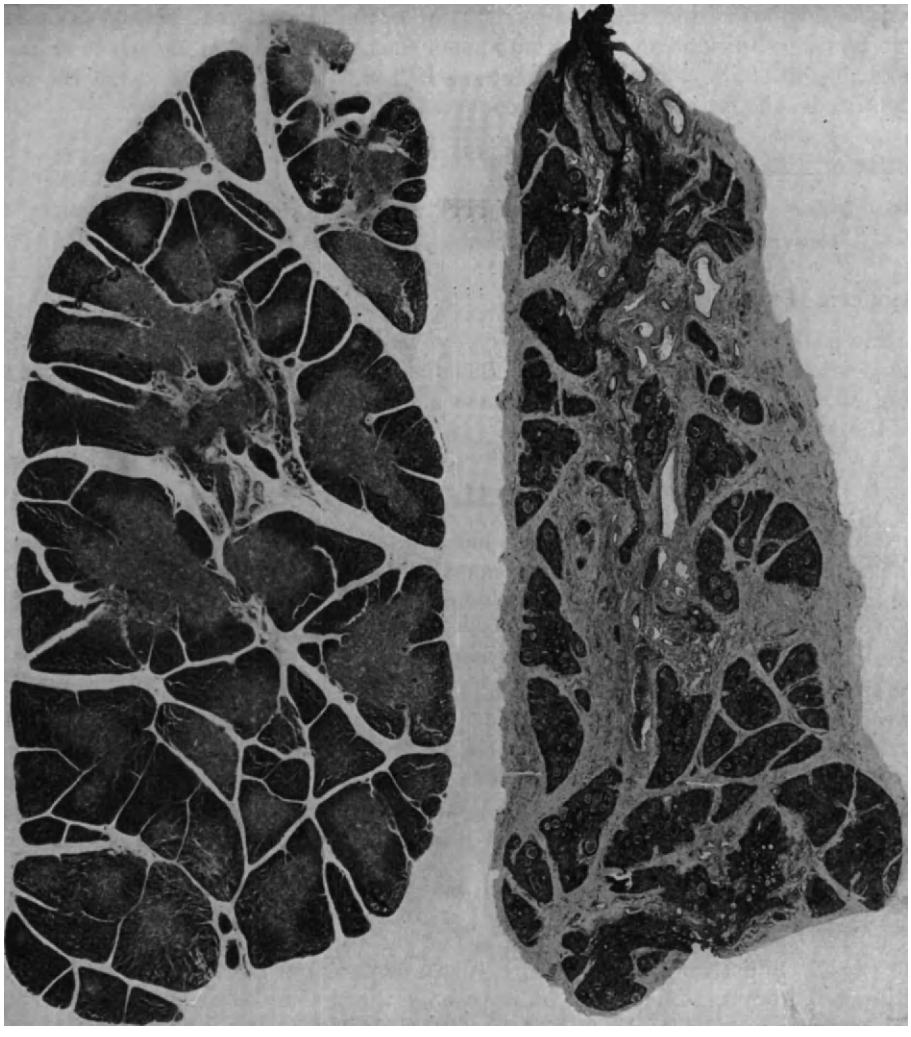


FIG. 14.10. A micrograph of sections of the thymus at two ages: from a new born child (left) and from a young adult when involution has begun (right). The lobular arrangement and the great increase in the connective tissue during involution is well shown. 1 mm.

Variations in Structure under Different Conditions

(1) Age

A well-defined cortex and medulla is seen up till the age of puberty, after which *involution* begins (Fig. 14.10). This process consists in a gradual diminution in the numbers of the lymphoid cells, a compression of the epithelio-reticular cells, and a replacement of these elements by adipose connective tissue derived from the interlobular septa. In the early stages of involution the Hassall corpuscles appear relatively large and numerous: ultimately they are nearly all replaced by the adipose tissue. This process continues normally throughout life.

(2) Accidental Involution

The thymus is never found in a normal condition in persons who have died of disease, as it responds with great rapidity to any adverse conditions. Usually it undergoes a very rapid and sudden involution due to a great emigration of its lymphocytes: this occurs particularly in acute febrile conditions, in starvation and in wasting diseases. Under these circumstances haemorrhages into the gland are common, and there is often an increase in the number of cosinophil cells: the reticular cells may contain ingested lymphocytes.

(3) Action of X-Rays

The effect of X-ray treatment is primarily the destruction of the lymphocytes. As a secondary result the epithelio-reticular cells proliferate.

(4) Lymphoid Hyperplasia

Certain pathological conditions (e.g. thyrotoxicosis and general lymphoid hyperplasia) are often associated with a persistent and usually enlarged thymus. In myasthenia gravis, a disease affecting neuromuscular function, it is sometimes hyperplastic, and its removal may then benefit the condition.

THE SPLEEN

The spleen is the largest haemolymph organ in the body and a large part of the reticulo-endothelial tissue of the body is concentrated in it. Its general structure is like that of the haemolymph nodes, the organ being closely connected with the blood and with the lymphoid tissue: its peculiarities depend on its specialized vascular arrangements and on the very large numbers of macrophages that it contains.

The spleen is covered, except where it is attached, by the *peritoneum*: immediately beneath this is the *capsule*, from whose inner surface project the *trabeculae* (or septa) that extend into the substance of the organ. The trabeculae, carrying the larger blood vessels, branch and anastomose, and are ultimately continuous with the branching reticular fibres and cells within the gland substance. The space between the trabeculae is filled with lymphatic tissue, the *splenic pulp*: the *white pulp* is distributed in roughly spherical or fusiform masses, the *red pulp* occupies the remaining space.

Capsule, Trabeculae and Reticular Framework

The capsule and trabeculae consist of dense connective tissue containing collagen and elastic fibres, fibrocytes and smooth muscle fibres: the thickest elastic fibres lie deeply in the capsule, and are most numerous in the trabeculae. The collagen fibres of the trabeculae continue directly into the reticular fibres constituting the framework of the red and the white pulp. The reticular fibres are everywhere associated with typical reticular cells of the reticulo-endothelial system.

White Pulp (Fig. 14.11, 14.13)

The white pulp consists of ordinary lymphoid tissue, i.e. a stroma of reticular fibres and reticular cells, with lymphocytes filling the interstices. It is gathered into fusiform or cylindrical masses (formerly known as *Malpighian corpuscles*) that surround the smaller arteries or arterioles. Frequently a germinal centre can be seen, consisting of dividing, large-type, lymphocytes.

In any section of the spleen, the centre of many of the cylinders of white pulp may be seen to be occupied by the central arteriole; this is a valuable diagnostic feature to remember.

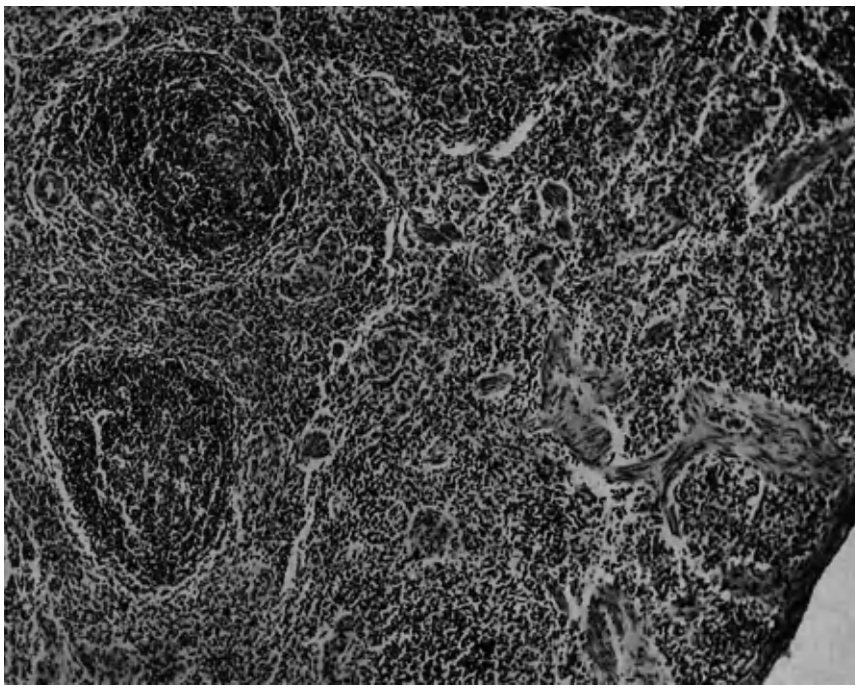


FIG. 14.11. A micrograph of a section of the spleen. The capsule is at the lower right hand side of the picture; some fibrous trabeculae can be seen in the substance of the organ near the capsule. Two cross-sections of cylinders of the white pulp are visible at the left of the micrograph. 300 μm.

Red Pulp (Figs. 14.12, 14.13)

The red pulp occupies the space between the large terminal venous sinuses that is not already occupied by the trabeculae and lymphoid cells of the white pulp: it was formerly called the "splenic" or "Billroth cords". The red pulp is primarily a reticular meshwork, honeycombed by venous sinuses. The non-granular leucocytes are very numerous, including cells of all sizes. Large, amoeboid phagocytic *splenic cells* (macrophages) are also present, and frequently contain engulfed particles. Granular leucocytes and erythrocytes are also found in the meshes of the stroma. *Giant cells* are sometimes present. Of the cellular elements in the red pulp there are therefore two types belonging to the reticulo-endothelial series, namely, the reticular cells of the stroma and the macrophages.

Blood Vessels

The structure of the spleen and the relation between the red and the white pulp depends on the arrangement of the blood vessels.

The arteries enter at the hilum and are distributed at first along the trabeculae, together with the large veins: they are typical arteries of the muscular type. The capsule has no separate blood supply. After much division the arteries leave the branches of the trabeculae and the veins and proceed alone, their adventitial coat becoming infiltrated with lymphocytes. This lymphoid tissue surrounds the arteries as a sheath almost up to the capillaries; in places the sheath is swollen into the cylinders of white pulp. In a transverse section the artery is seen to be placed excentrically in the corpuscle, the germinal centre (if present) occupying the middle (Fig. 14.13). As the swelling is often in the neighbourhood of a branching of the artery, a section may show more than one blood vessel within a section of the white pulp. A few capillaries are given off to the white pulp, and the artery then breaks up into a tuft of straight

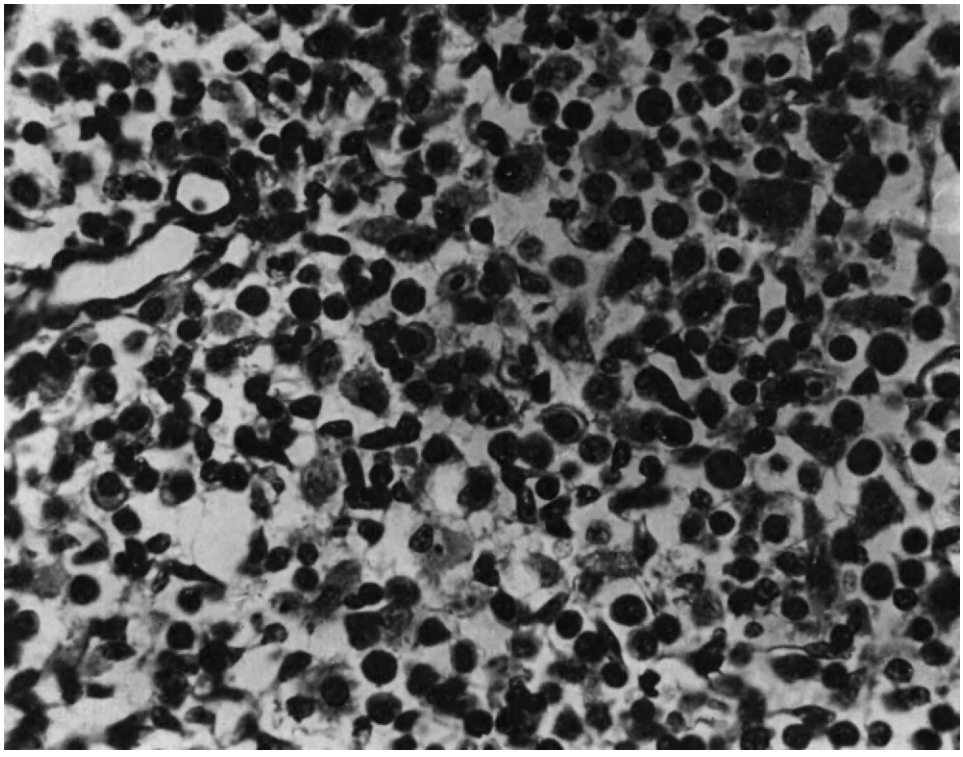


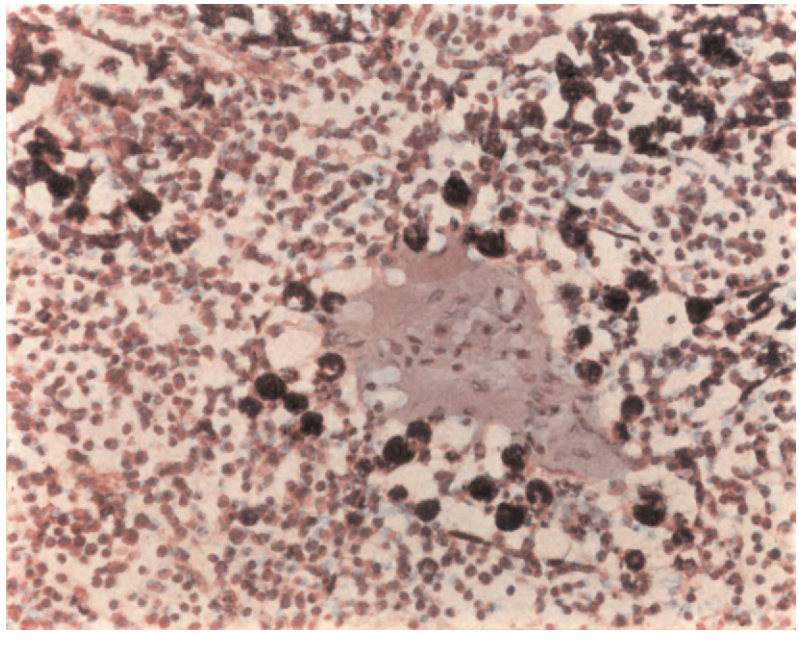
FIG. 14.12. A high power micrograph of the red pulp of the spleen. 40 μm.

arterioles, the *penicilli*. These arterioles pass into the red pulp dividing into capillaries that are invested in a special sheath of concentric lamellae or connective tissue infiltrated with lymphocytes and reticulo-endothelial cells: this is the *ellipsoid*. The arterial capillary leaves the ellipsoid, and is expanded into an ampulla whose walls blend with the pulp cells: the blood thus flows out into the splenic pulp. After wandering through the pulp, the blood is collected into the venous sinuses. The ellipsoid acts as a valve, preventing any back flow of blood from the venous sinuses, or too rapid a flow from the arterial side and also acts as a filtration bed for the blood.

The *venous sinuses* form an anastomosing network penetrating the whole of the red pulp, and draining away into the veins. The sinus has a wide lumen, varying greatly with the blood content of the spleen. It is lined by actively phagocytic macrophages, supported externally by

PLATE 2

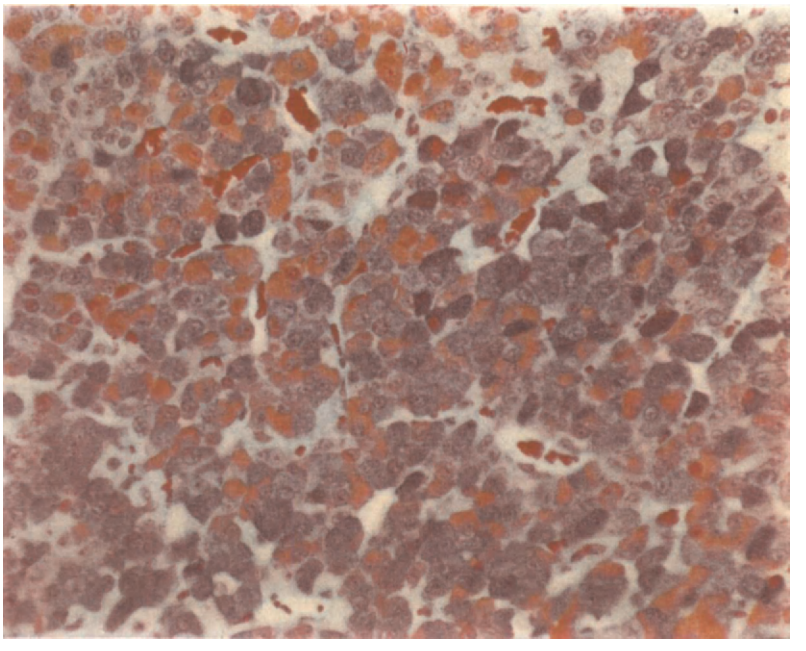
A. A photograph of the areolar connective tissue on the undersurface of the skin covering the leg of a mouse. The animal had received an injection of blue dye into the foot pad a few minutes previously. Note the lymphatic vessel (coloured blue) running across the picture, in company with blood vessels.



B. Phagocytic reticulo-endothelial cells in a lymph node. These are the large round cells shown up here by virtue of the dye particles which they have ingested and accumulated within their cytoplasm.

[*To face p.* 212.

PLATE 3



The appearance of the adenohypophysis following staining by a standard trichrome procedure.

The red blood corpuscles in the sinusoids appear bright red, whilst the basiphils are coloured bluish-purple and the acidophils are orange. Chromophobe cells (with unstained cytoplasm) may be seen scattered throughout the field, especially at the upper left of the picture.

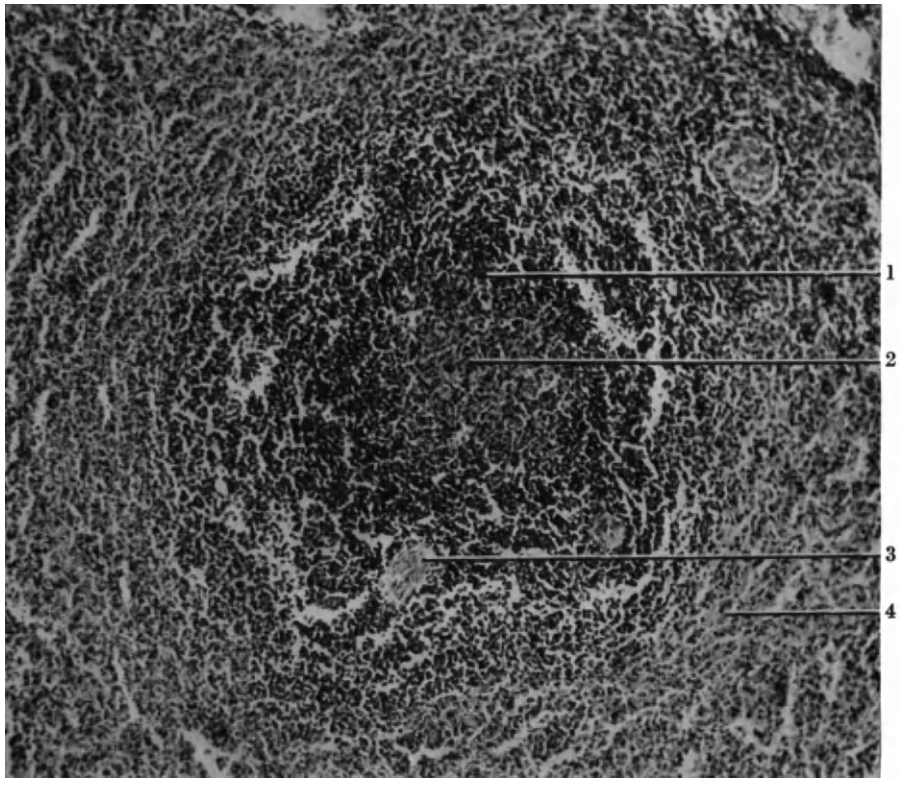


FIG. 14.13. Part of the peripheral tissue of the spleen. 1, white pulp. 2, germinal centre. 3, arteriole entering the white pulp. 4, red pulp. 150 μm.

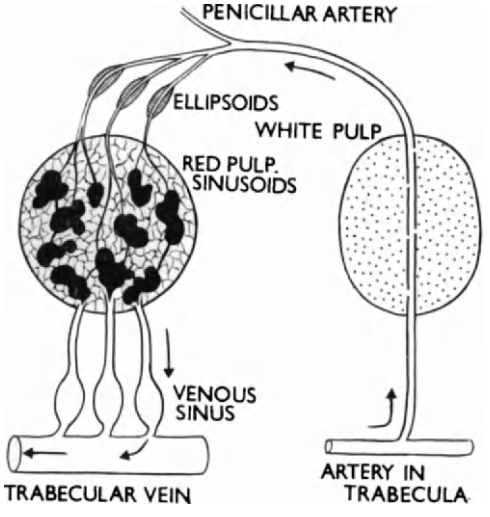


FIG. 14.14. A diagram of the main features of the splenic blood circulation.

reticulin fibres: there are small slit-like openings through which blood cells pass freely. The lining cells are dilated in the regions occupied by their nuclei. The venous sinuses empty into the *veins*, which ultimately join to run in the trabeculae: the venous wall consists of endothelium directly touching the fibres of the trabeculae. The general pattern of the circulation is summarized in Fig. 14.14.

Union of Arteries and Veins

The different views in relation to the circulation of the blood in the spleen result, in part, from the fact that there are considerable species differences. In the mouse, for example, which has been extensively used in investigations on the splenic circulation, there are no venous sinuses such as those described above. Much of the earlier work which claimed that the splenic circulation was "closed", that is that the arterial capillaries open directly into venous sinuses, was based on this animal. It must now be presumed that small veins rather than sinuses were observed. Although the subject is still controversial, there is increasing evidence, obtained from other species, that the capillaries open directly into the meshwork of the red pulp by means of funnel-shaped openings. This "open" type of circulation provides, in the meshes of the red pulp, a mechanism for the separation of red cells from plasma.

Lymph Vessels

The actual pulp tissue of the spleen possesses no lymphatics, the tissue fluid passing directly into the venous sinuses. Lymphatic vessels are found in the largest trabeculae and in the capsule: near the hilum these anastomose and leave the organ in company with the blood vessels.

Nerves

Most of the nerve fibres are non-myelinated. They are post-ganglionic sympathetic fibres derived from the coeliac plexus, and enter at the hilum with the splenic artery and vein. The bundles accompany the arteries in all their ramifications, and ultimately form networks that provide bulbous endings in the smooth muscle of both arteries and trabeculae. The musculature of the splenic vein and its branches is similarly provided with non-myelinated vasomotor fibres. A few myelinated fibres, probably afferent in nature, are also present in the spleen.

Functions of the Spleen

The large amount of reticulo-endothelial tissue in the spleen is of importance for the assimilation of the fragments of red blood corpuscles. These fragments are phagocytosed by the macrophages and the iron moeity of the haemoglobin is freed from the stroma of the destroyed red cell and stored as haemosiderin in the reticulo-endothelial cells until it is required by the body for the resynthesis of fresh haemoglobin.

Many pyroninophil cells (probably plasma cells) are found in the red pulp of the spleen and hence this organ in conjunction with the lymph nodes and thymus is an important site of antibody manufacture.

Because of its large content of lymphoid tissue, the spleen must be regarded as an important centre of lymphopoesis, the lymphocytes and monocytes being produced in the white pulp and then migrating to the blood stream via the red pulp. In some species other types of blood cell are produced in the spleen, e.g. granulocytes and the platelets. This is not so in man. Again, in other species it has been reported that the spleen may act as a reservoir for red blood corpuscles; this may be only of secondary importance in humans.

Variations in the Microscopic Structure of the Spleen

(1) Age

During embryonic and foetal development the spleen tissue is not divisible into red and white pulp; its function is chiefly haemopoietic, and all the types of red and white blood

corpuscles are formed here. After the first few post-natal weeks the myeloid elements gradually disappear, the adventitia of the arteries is infiltrated with lymphocytes and the white pulp is defined. Haemopoiesis stops, with the exception of lymphocyte production in the germinal nodes of the white pulp, and the organ takes on an erythrolytic function. But the white and the red pulp consist primarily of the same tissue whose cells retain a haemopoietic potentiality throughout life.

As age advances the germinal centres of the white pulp gradually disappear and the trabecular tissue becomes more prominent: the whole organ tends to atrophy.

The giant cells are most often seen in the spleens of young children.

(2) Activity

The spleen undergoes small variations in size due in part to contraction and relaxation of the smooth muscle of the capsule and trabeculae, but due also in greater measure to variations in the amount of blood that it contains: this latter factor is controlled by the nerves supplying the walls of the veins and of the arteries. When the veins are contracted the venous sinuses become engorged with blood.

During digestion the spleen increases in size: the reason for this is unknown. It undergoes a rhythmic contraction about once a minute: this is probably connected with the need for aiding the blood circulation through the sinuses. Variations in the size of the spleen are also associated with its function of acting as a temporary storehouse for erythrocytes. During starvation the white pulp diminishes in amount and the whole organ shrinks. The phagocytic functions of the spleen, and its production of antibodies, are of primary importance, and dependent on the close relation existing between the pulp cells and the circulating blood: the relation is similar in the liver and in the bone marrow, which share these functions, the spleen macrophages being the most active, and those of the liver ranking next.

(3) Response to Various Conditions

A general leukaemia is frequently associated with an enlarged spleen, the pulp becoming packed with leucocytes of the type characterizing the blood: in the myeloid type the cells are chiefly myeloblasts, myelocytes and granular leucocytes, while in the lymphatic type the cells are mostly lymphocytes.

Many infections are characterized by an enlarged spleen; organisms of various kinds may accumulate in the pulp, and the reaction invoked for their destruction is responsible for the enlargement of the organ. The slowing down of the blood stream as it passes through the pulp allows the phagocytic elements to exercise their activity, a mechanism which is aided further by the engorgement with blood that is brought about by the action of the toxins on the controlling vasomotor nerve fibres.

In some severe anaemias (such as that associated with malignant growth in bone), the spleen may resume haemopoietic activity: this potentiality remains throughout life.

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CHAPTER 15

THE ADRENALS, THYROID AND PARATHYROID GLANDS

All the organs considered in this and the following chapter are *endocrine glands*. These are characterized principally by the fact that their secretions, the *hormones*, leave the gland not by a duct but by the blood stream, which then distributes them to their site of action. This may be a single type of target cell or, as in the case of thyroid hormone, it may be a large number of different cell types. All the endocrine glands possess a very rich blood supply (see Fig. 13.9), an adaptation suggestive of ease of transfer of product from gland cell to blood.

ADRENALS

The paired adrenal (suprarenal) glands are found in the retroperitoneal adipose tissue; they are shaped like cocked hats and are closely applied to the cranial pole of each kidney. In section they are roughly triangular, with a definite hilum on the anterior surface.

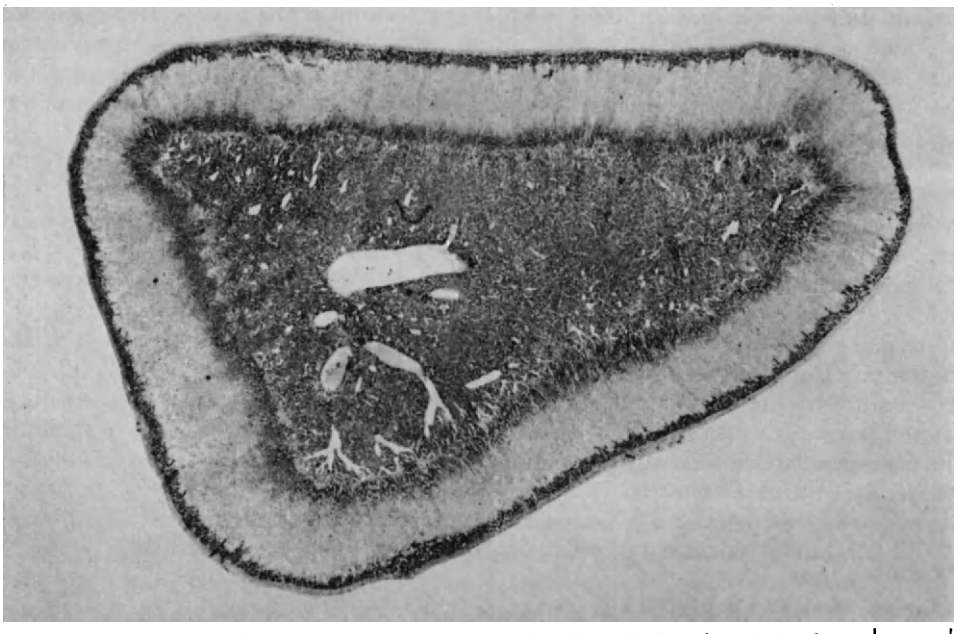

FIG. 15.1. A low power micrograph of a transverse section through the adrenal gland. The cortex, with the darkly staining zona glomerulosa (just below the capsule) and the medulla are clearly visible. Note the large central vein.

1 mm.

When quite fresh the cut surface of the gland shows a firm orange-yellow *cortex* surrounding a soft, reddish *medulla* (Fig. 15.1), the latter being very vascular. If the gland has been fixed in potassium dichromate the cut surface then presents the picture of a pale cortex and a yellow-brown medulla. This so-called *chromaffin reaction* of the medulla (Fig. 15.4) is due to the presence of adrenalin in the medullary cells. The adrenalin will also give a green colour with ferric chloride.

Capsule

The gland is enclosed in a thick capsule of fibrous connective tissue containing a few muscle fibres. The outermost part of the capsule is loosely arranged and merges with the surrounding areolar tissue. The capsule sends fine trabeculae into the organ, providing a framework and dividing the glandular tissue into compartments.

Cortex

The cells are arranged in three somewhat vaguely defined layers that merge into one another—an outer zona glomerulosa, a middle zona fasciculata, and an inner zona reticularis (Fig. 15.2). The arrangement of the cells in the different layers varies in relation to the blood vessels.

FIG. 15.2. A micrograph of the adrenal cortex. The zona glomerulosa is at the upper right of the picture whilst the zona fasciculata occupies most of the centre. The zona reticularis and a few cells of the medulla may be seen at the bottom left of the micrograph.

In the narrow *zona glomerulosa*, immediately beneath the capsule (which is not so well defined in man as it is in some other animals) the cells are small, columnar and packed closely together in rounded groups, and the free surface of each cell adjoins a capillary. The nuclei stain deeply and the cytoplasm is basiphil. It appears that *aldosterone* and *deoxycorticosterone* are produced predominantly by this layer, which is largely independent of hypophysial control. Aldosterone promotes resorption of sodium ions by the proximal convoluted tubule cells of the kidney in exchange for potassium and hydrogen ions whose excretion is enhanced.

The *zona fasciculata* is the thickest layer of the cortex. The cells are large, polyhedral with vesicular nuclei, and very rich in lipid, the latter being demonstrable as spherical droplets after appropriate treatment with Sudan IV or osmium tetroxide. In the fresh condition this lipid gives the cortex its yellow colour: in the usual histological section the lipid has been

dissolved out and the cells appear vacuolated. In addition to the lipid droplets this zone of the cortex is particularly rich in cholesterol and vitamin C. Following upon stimulation of the cortex by, for example, the adrenocorticotrophic hormone (A.C.T.H.) of the pituitary, there is a marked depletion of both of these substances. The cells are arranged in columns, with strands of connective tissue and capillaries running between the columns. This zone is directly influenced by pituitary A.C.T.H. and produces most of the *glucocorticoids* (*cortisone* and *cortisol*) which participate in carbohydrate, protein and fat metabolism. Sex hormones (*oestrogens* and *androgens*) also appear to be produced in small amounts by the two inner zones of the adrenal cortex.

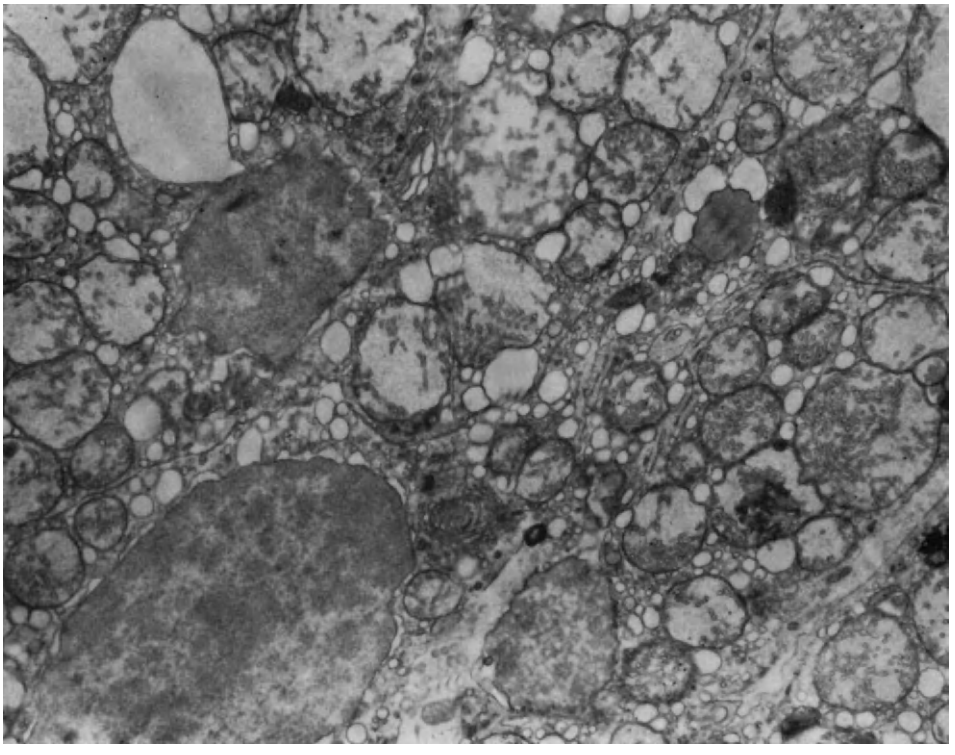

FIG. 15.3. An E/M of the cells of the zona fasciculata of the adrenal cortex. Preparation fixed in hydroxyadipaldehyde. Note the presence of numerous lipid droplets, some with their contents partly dissolved by the preparative techniques, mitochondria with tubular cristae and vesicles of the smooth endoplasmic reticulum. The nucleus of the cell, with the Golgi apparatus close to it, is at the bottom left of the micrograph. 1 μm.

The E/M shows that the cells of both the zona glomerulosa and the zona fasciculata of the cortex are characterized by the presence of large amounts of smooth surfaced endoplasmic reticulum which forms an anastomosing network of tubules throughout the cytoplasm. Some free ribosomes are present; in the zona glomerulosa the Golgi apparatus is small, but it is much more prominent in the cells of the zona fasciculata. In this latter zone the mitochondria differ from those in other organs in that they have long tubular or vesicular cristae rather than the plate-like form which is more usual (Fig. 15.3).

In the *zona reticularis* the cords of cells are arranged as an anastomosing network round the sinusoids into which the straight capillaries of the zona fasciculata merge. The cells are like those of the fasciculata layer but contain less lipid. The evidence suggests that they also

produce glucocorticoids. The innermost layer often contains degenerating cells, and large "light" cells can be distinguished from small "dark" cells. Pigment is sometimes present.

There is some evidence for the belief that new cells are formed by mitosis particularly in the junction region between the z. glomerulosa and the z. fasciculata. This would imply a gradual centripetal "drift" of cells towards the z. reticularis, where presumably they ultimately degenerate forming the cells mentioned above. The degenerate cells are removed by macrophages.

The boundary between cortex and medulla is irregular.

In infants, very small nodules of accessory cortical tissue are often present chiefly in the retro-peritoneal tissue, the testis and the ovary: these nodules rarely persist into adult life.

The adrenal cortex is essential for life; it is concerned with controlling the maintenance of fluid and electrolyte balance in the body, with the maintenance of carbohydrate balance and with the integrity and correct functioning of some of the cells and other components of the connective tissue. In man, removal or destruction of the cortex leads to the development of Addison's disease and ultimately death (unless synthetic cortical hormones are given). There is excessive excretion of sodium ions in the urine and a consequent lowering of the plasma sodium concentration. At the same time there is a fall in the plasma concentrations of chloride and bicarbonate ions. Tissue dehydration ensues and a rise in the concentration of the blood plasma. Hypoglycaemia (a fall in the blood sugar content) and a loss of the glycogen stores in the liver and in the muscles would also be apparent.

Further details of the organization and function of the adrenal cortex may be found in the article by Dobbie, MacKay and Symington listed in the references.

Medulla

The medulla consists of polyhedral cells arranged in groups that are in contact all round with venous sinuses. The cells, which are supported by a meshwork of fine fibres, contain small granules that give the chromaffin reaction symptomatic of the oxidation of the catecholamines adrenalin and noradrenalin (Fig. 15.4). In man there is about ten times more adrenalin than noradrenalin in the medulla.

The E/M shows that the chromaffin granules are electron-dense and bounded by a membrane. Some of the granules show a much higher degree of electron-density and these are believed to be those containing noradrenalin. Recent electron histochemical procedures have been developed by Woods and Barrnett, among others, which allow the two compounds to be differentiated at the E/M level with certainty.

In addition to the granule-containing cells, a few sympathetic ganglion cells may be recognized as such; the current view is, however, that the granule-containing cells are themselves homologous with the sympathetic ganglion cells. As such, the activity of the adrenal medulla is largely influenced by the activity of nervous centres in the hypothalamus of the brain, which passes impulses to the gland via the preganglionic sympathetic fibres in the splanchnic nerves.

The adrenal medulla is not essential for the maintenance of life, although its removal makes the animal less able to cope with emergency demands. The secretion of adrenalin causes an increase in the heart rate and in its output (with, however, no significant increase in the blood pressure); it also increases the level of the basal metabolism, elevates the blood sugar level, increases the oxygen consumption. Noradrenalin, on the other hand, has little effect on the heart rate or output or on the metabolic rate but it does elevate the blood pressure by vasoconstriction. Both hormones affect the process of the release of fat from adipose tissue by increasing the rate of lipolysis.

Blood Vessels

The arteries, usually three in number, enter at the surface and branch in the capsule: a few branches then pass straight through into the medulla, but the remainder supply capillaries

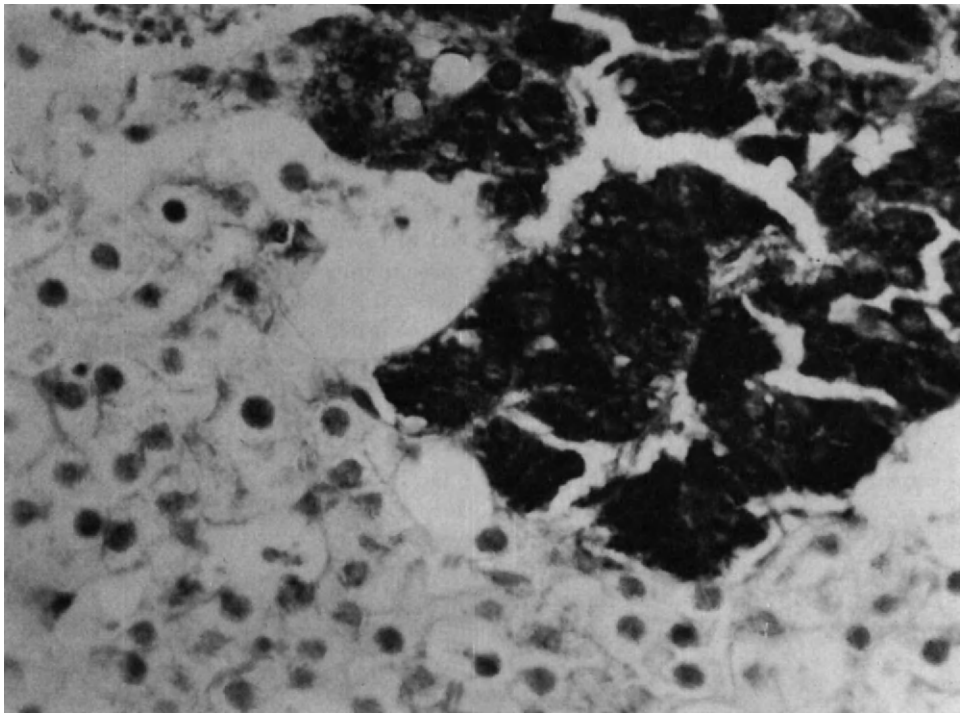

FIG. 15.4. The adrenal medullary/cortical junction prepared by a technique which reveals catecholamines (shown here darkly stained) in the medullary cells. Note that there is no reaction in the cortical cells.

20 μm.

for the cortex. The capillaries run in the fibrous septa between the cell columns and empty into the sinusoids of the zona reticularis and the medulla. The sinusoids drain into venules which, in turn, empty into larger veins; these unite and emerge at the hilum as one or two large veins.

The larger veins have remarkably thick walls containing much smooth muscle: this is mostly arranged in longitudinal bundles unevenly distributed in the wall and projecting into the lumen. The sinusoids are lined partly by endothelium and partly by macrophages: between these cells and the glandular cells is a network of reticulin fibres. The structure of the sinusoids resembles that of the liver vessels.

Lymphatic Vessels

A very rich lymphatic plexus has been described in the glandular substance. There is also a superficial lymphatic plexus, a deep capsular plexus, a plexus draining from the medulla, and lymphatic vessels in relation to the large veins.

Nerves

The capsule contains a plexus of nerve fibres mostly non-myelinated, and scattered ganglion cells of the sympathetic system. The plexus sends fine fibres into the cortex, where they end as networks round groups of cells. In addition, bundles of (myelinated) pre-ganglionic fibres pass to the medulla, where they form dense plexuses and end by surrounding the individual cells: these cells are homologous to the ganglion cells and post-ganglionic fibres. Some of the fibres are related to the small groups of sympathetic ganglion cells that are present in the medulla.

Development

The cortex and the medulla have different origins. The medulla is derived from the neural crest tissue (which also forms the sympathetic ganglion cells): groups of cells separate off from the rudimentary sympathetic ganglia and migrate to the neighbourhood of the developing cortical mass, which they begin to enter on its medial side at about the 12 mm. stage of embryonic development. The immigration is most active between the twelfth and the twenty-second week of development and ceases at birth. During migration the cells are chiefly small, round and darkly staining: they come to rest round the large central blood vessels and slowly differentiate into the typical medulla cells. Any nerve cells present in the gland have been carried in with nerve fibres during the immigration period.

The cortex consists of two parts, a *foetal cortex*, that constitutes the bulk of the gland in the foetus and degenerates after birth, and a *true cortex*, which is small in foetal life but persists and gives rise to the post-natal cortex. The foetal cortex is derived from the coelomic epithelium over the mesonephros, the true cortex from that of the genital ridge. The true cortex appears as a narrow rim of small basiphil cells immediately beneath the capsule: during the last ten weeks of foetal life it increases by downgrowth into the mass of the gland, and at birth a columnar arrangement of the cells is apparent. The foetal cortex (or "boundary zone") that makes the bulk of the foetal gland consists of chains of large, granular, acidophil cells with a rich capillary supply. During the last two or three months of foetal life the foetal cortex begins to degenerate, the process being much accelerated during the last two weeks: by the end of the first year of life it has disappeared entirely and its place is taken by the downgrowing cords of true cortex.

Variations under Different Conditions

(1) Age

As explained above, at birth the adrenal gland consists of a capsule enclosing a true cortex that is growing rapidly inwards to replace the large mass of degenerating foetal cortex: the centre of the gland is occupied by a thin zone of medullary tissue. As the foetal cortex disappears the true cortex contracts on the medulla, so that the gland becomes much smaller than it was during foetal life. (In an eight weeks' foetus the kidney and the suprarenal are about the same size (Fig. 15.5): at birth the adrenal is a third of the size of the kidney, and in the adult only about one-thirtieth of its size.) By three years of age the three cortical zones can be distinguished.

After puberty the zona reticularis contains yellow-brown "lipochrome" pigment that gradually increases in amount with age (this is the same pigment that appears in cardiac muscle cells and in nerve cells in later life).

(2) Varying Physiological Conditions

In fatigue the adrenalin, as identified by the chromaffin reaction, disappears from the peripheral zone of the medulla. This also happens in conditions of increased thyroid activity and under any circumstances that cause prolonged sympathetic stimulation. Exercise and cold, especially if acting simultaneously, deplete the adrenalin store, as do also anaesthetics.

During pregnancy the cortex hypertrophies and its lipid content is increased. Stress depletes the cortex of cholesterol and vitamin C.

(3) Various Pathological Conditions

The suprarenal glands frequently manifest hypertrophic changes when any of the other ductless glands are functioning abnormally. Foetal acephaly is associated with abnormality of the suprarenals. Haemorrhages are comparatively common, and the medulla is sometimes

almost replaced by blood and blood clot, with only a thin covering of cortex remaining outside.

The normally occurring destruction and proliferation of cells of the cortex is greatly accelerated by acute infections, blood toxins and prolonged narcosis. The blood flow through the sinusoids of the zona reticularis is slow and the cells are thus exposed to the blood for a long time: this explains why evidence of damage is most readily seen in this layer. The lipids

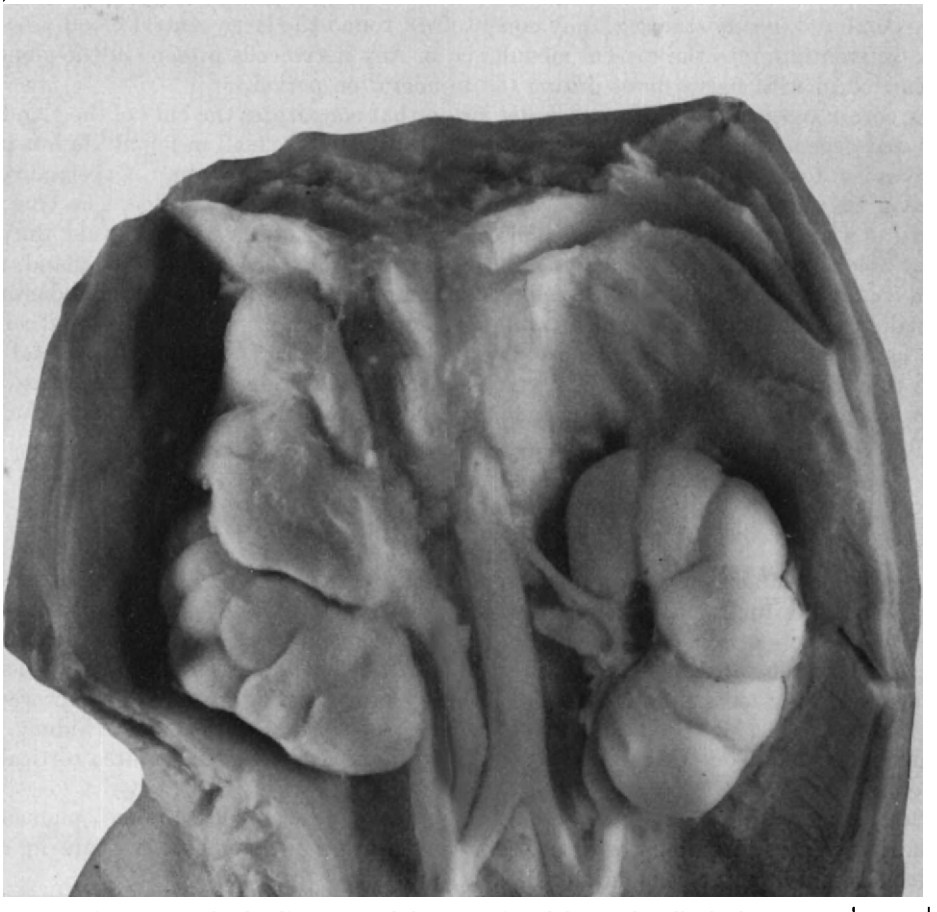

FIG. 15.5. A photograph of a dissection of the posterior abdominal wall of a human foetus. The two lobulated kidneys may be seen, and above the right kidney of the foetus (i.e. on the *left* of the photograph) is the large, pyramidal-shaped adrenal. The adrenal on the other side has been removed in the dissection.

in the cortical cells disappear in acute infectious diseases, but they are not decreased in starvation.

Atrophy of the cortex, or extensive destruction due to tubercular infection, is often associated with bronzing of the skin as in Addison's disease: the origin of the pigment is not definitely known. In Addison's disease the medulla is often affected as well as the cortex.

THE PARAGANGLIA

The term paraganglia includes various scattered collections of cells that are closely associated anatomically and embryologically with the sympathetic system. They occur in

association with the sympathetic ganglia, and in appearance and staining reactions they are similar to the cells of the adrenal medulla (see p. 219). The cells are large, and epithelial-like, arranged in cords in relation to many small blood vessels and numerous nerve endings. Because they give the chromaffin reaction it has been assumed (but not proved) that the cells secrete adrenalin. These groups of cells are chiefly retroperitoneal, and include the *organs of Zuckerkandl* (well-marked in foetal life) at the level of bifurcation of the abdominal aorta, and similar collections of cells in the kidney, ovary, testis, and heart (in the connective tissue between the aorta and the pulmonary artery).

The *coccygeal body* (formerly included in the group of paraganglia) is probably a glomus (see p. 197).

The *carotid and aortic bodies* (also formerly included as paraganglia) are concerned with reflexes, depending on the chemical composition and pressure of the blood.

THYROID GLAND

The thyroid gland in the anterior part of the neck is concerned with the production, storage and release into the blood stream of the hormones *thyroxine* (T_4) and *tri-iodothyronine* (T_3) which mediate the rate of the basal metabolism of the body. The thyroid gland also secretes

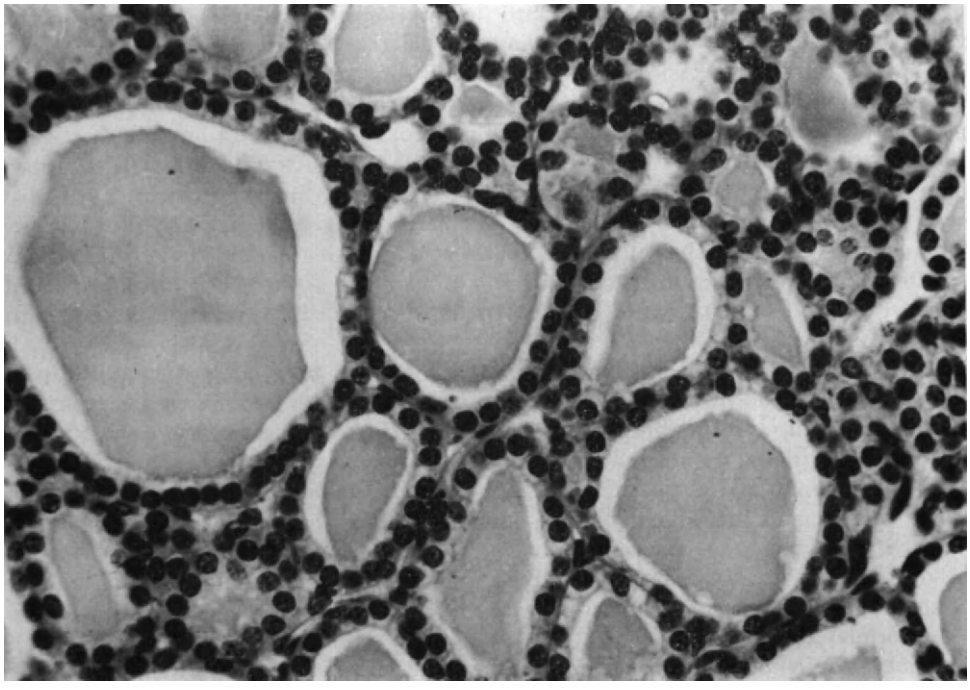

FIG. 15.6. A micrograph of follicles of the thyroid gland. Many of the follicles are cut in tangential section. Note their typical cuboidal epithelium and the coagulated colloid which has shrunken away from the follicle wall. 30 μm.

another hormone, *calcitonin*, which influences plasma calcium level, causing the latter to fall, and which inhibits bone resorption by osteoclasts. The gland itself usually consists of two lateral lobes joined by an isthmus. Each lobe is surrounded by a dense connective tissue capsule consisting of two layers: the external layer is continuous with the surrounding cervical fascia, and is loosely connected with the inner layer, which is dense and closely adherent to the organ.

The connective tissue capsule is continuous with the framework of the gland: it appears as masses of connective tissue arranged as irregular interconnecting bands that separate the lobules of glandular tissue (or parenchyma) from one another. The parenchyma consists of groups of irregularly spherical follicles separated from one another by reticular connective tissue carrying blood vessels, lymphatics and nerves (Fig. 15.6).

The Follicles

As the follicles are for the most part spherical they all appear more or less round in cross section. They are lined by epithelial cells which are usually cubical with spherical nuclei: they contain granules and droplets, probably of a secretory nature. The follicular surfaces of the cells contain variable numbers of microvilli shown in the electron micrograph (Fig. 15.7A). These increase in length and in numbers when secretion is activated. The E/M shows that the thyroid follicle cells possess a large quantity of rough-surfaced endoplasmic reticulum. The Golgi apparatus is well developed and the apical cytoplasm is characterized by the presence of numerous membrane-bound dense granules which probably represent lysosomes. It is sometimes stated that a basal lamina is absent, but this appears to be true only of the young condition. The presence of a basal lamina is certainly revealed by the electron microscope in the thyroids of mature animals. The Golgi apparatus of these cells is almost invariably situated between the nucleus and the lumen of the follicle, a fact which strongly suggests that the specific secretion is initially liberated into the interior of the follicles. This view is further supported by direct observations upon living thyroid tissue grown in transparent chambers within the ears of rabbits. Experiments of this kind suggest a "two-way traffic". Firstly, the secretion of a substance into the follicular lumen (the "colloid" of fixed material) and secondly the passage outwards, across the cells, of this intrafollicular material (or a derivative of it) directly into the blood vessels surrounding each follicle. The thyroid would thus appear to combine both exocrine and endocrine secretory mechanisms, but the exocrine mechanism is atypical in that the secretion is discharged into a series of closed spaces and thus has secondarily to be conveyed across the cells again to be discharged into the blood stream. Perhaps the initial exocrine mode of secretion is a relic from a time in the phylogenetic history of chordates when the equivalent of the thyroid gland normally discharged its secretion directly into the alimentary canal.

The lumen of the follicle is usually filled with colloid material (a PAS-positive glycoprotein called *thyroglobulin*) which binds the hormones T_3 and T_4. Similar material can sometimes be demonstrated in the lymphatics and even in the interstices of the connective tissue. The colloid is coagulated by alcohol and shrinks in fixing, so that a small space often appears in sections between it and the cells of the alveolar wall. A proteolytic enzyme has been described as occurring in the colloid but its precise role is still a matter for speculation. The internal portion of the cells sometimes contains droplets having properties similar to those of the colloid. New follicles can be formed by a proliferation of the follicular epithelium, the secondary follicles later losing connection with the original follicle: such new follicles are not normally formed after puberty.

If animals are fed on a diet containing small quantities of radioactive iodine, the distribution of the iodine within the thyroid can be studied autoradiographically, although the resolution obtained is not very good; better results may be obtained by using a tritiated amino acid. Using these methods it may be shown that iodine very rapidly accumulates in the colloid of the thyroid follicles.

Interfollicular Tissue

The interfollicular connective tissue contains lymphocytes and macrophages. Groups of epithelial cells that do not surround a lumen are the walls of tangentially cut follicles, not a separate structure. Sometimes small portions of parathyroid tissue or of thymic tissue are found embedded in the gland.

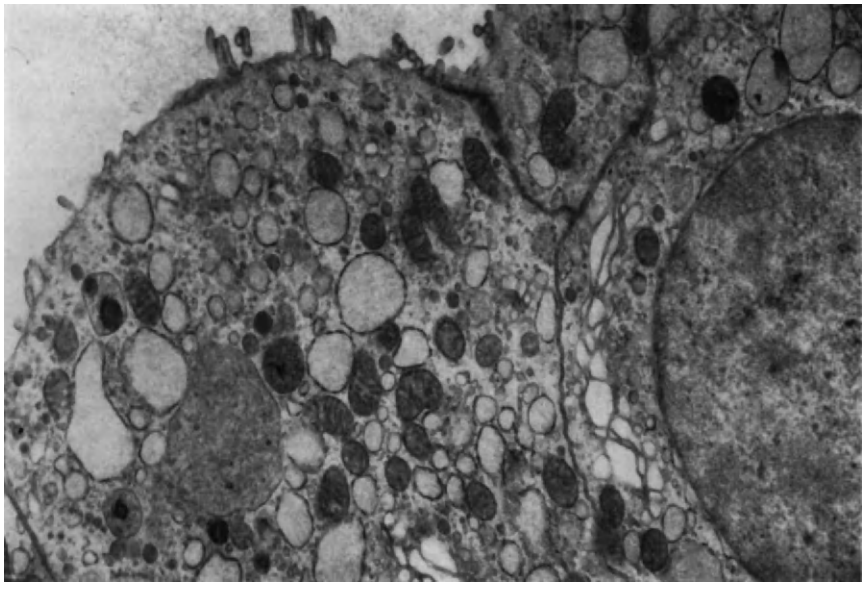

FIG. 15.7A. An E/M of thyroid follicle cells. This is a relatively quiescent cell; the colloid is at the upper left. Many mitochondria and dense granules may be seen as well as vesicles of endoplasmic reticulum. 2 μm.

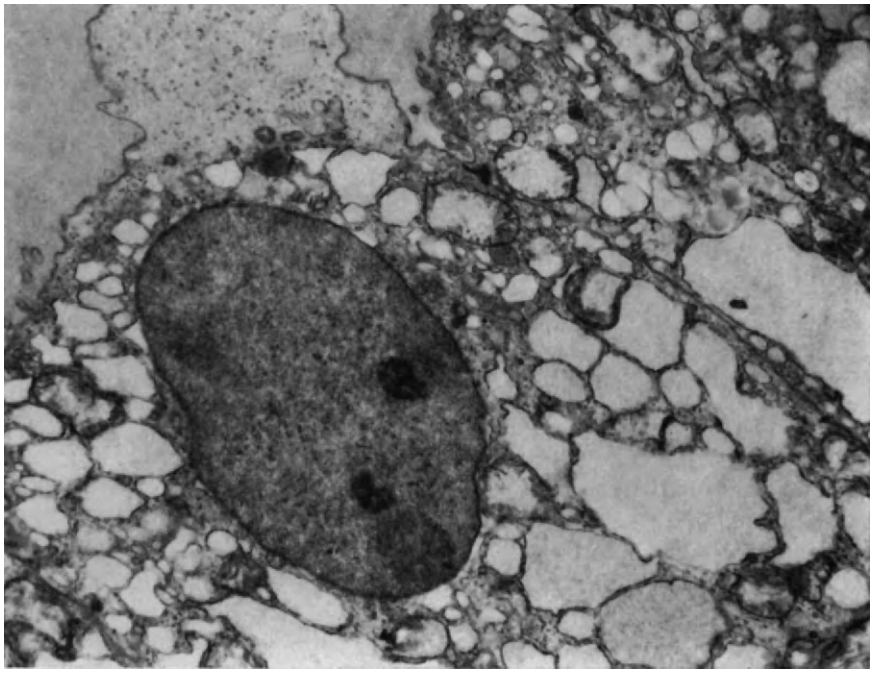

FIG. 15.7B. An active thyroid cell (following stimulation by an injection of long acting thyroid stimulating hormone). Note the "blebbing" of the apical cytoplasm into the follicle lumen and the great increase in the amount of the rough surfaced endoplasmic reticulum. Micrographs by courtesy of Dr. El Kabir. 2 μm.

Parafollicular Cells (C Cells)

These cells were first observed at the end of the nineteenth century and were named by Nonidez in 1931. They have recently been the subject of much investigation, using histochemical techniques and electron microscopy. They have been shown embryologically to be derived from the so-called *ultimo-branchial body* which occurs on the last pharyngeal pouch of the embryo.

The parafollicular cells are larger than the typical follicular cells and they have been shown to be histochemically quite distinct, containing in particular fluorogenic amines and high concentrations of the enzyme α glycerophosphate dehydrogenase. It now seems certain that these cells are the site of production of the hormone *calcitonin* which acts to lower the serum calcium level.

Blood Vessels

The thyroid gland is unusually vascular, the vessels running in the connective tissue partitions and ultimately forming a capillary network surrounding every follicle. The capillaries show prominent fenestrae in the endothelial cell walls. The collecting veins also run in the interfollicular connective tissue.

Lymphatics

The lymphatic drainage is very ample and free, the vessels accompanying the blood vessels. There is a dense sub-capsular lymphatic plexus. The lymphatics from the thyroid may join the adjacent internal jugular vein without interruption in any intervening lymph node: this accounts for the early blood spread of thyroid carcinoma.

Nerves

Most of the nerve fibres are sympathetic post-ganglionic and non-myelinated, derived from the middle and superior cervical ganglia, and entering with the blood vessels. They form plexuses in the walls of the blood vessels and are mainly vasomotor. A few end in networks round the follicles.

Development

The gland arises as a hollow median ventral outgrowth from the pharynx, at the point which is represented by the foramen caecum at the base of the tongue in the adult. The tube grows caudally, thickens at the end, and usually loses its connection with the mouth: occasionally the duct persists as the *thyroglossal duct*. The mass is at first solid and then splits into cords of epithelium, in which the primary follicles are hollowed out. The vascular mesoderm grows in to surround groups of cells. Colloid material appears in the follicles as early as the sixteenth week of foetal development, and iodine can be detected by eighteen weeks.

Variations in the Thyroid Gland in Response to Various Conditions

(1) Age and Functional Activity

The activity of the thyroid varies greatly at different ages, particularly in the female. It is not functionally adequate until towards the end of the first year, and assumes increased activity at puberty, and during menstruation, pregnancy and lactation: after the menopause it tends to atrophy. The control of the activity of the thyroid is largely regulated by the amount of thyrotrophic hormone (T.S.H.) secreted by the anterior lobe of the pituitary gland.

Increased activity of the gland is usually associated with a tall, columnar epithelium (Fig. 15.8), which may become folded to increase the secreting surface. There is usually little colloid, and vacuoles often occur round the periphery of such colloid masses as are present, indicating (in all probability) a resorption of the stored secretion in a soluble form. The E/M

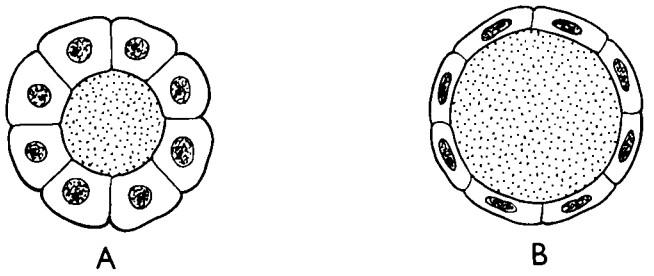

FIG. 15.8. A diagram of thyroid follicular cells A, just becoming active. B, inactive, with accumulation of large amounts of colloid.

shows that the rough surfaced endoplasmic reticulum undergoes much hypertrophy under these conditions of increased glandular activity. The microvilli on the luminal borders of the cells also increase in number and in length and may even become "bleb-like" (Fig. 15.7B). The basal aspect of the follicular cell membrane also shows marked infoldings. A predominance of tall, over-active epithelial cells (Fig. 15.7B) and a deficiency of colloid are characteristic of hyperthyroidism. In Graves' disease there is a great increase in the activity of the gland, and also a

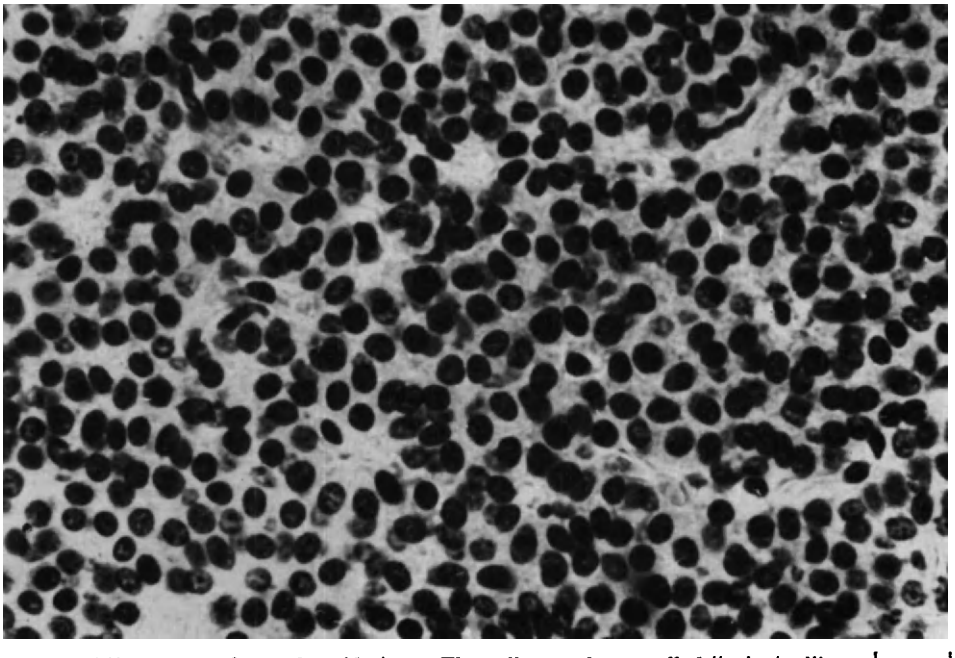

FIG. 15.9. Micrograph of parathyroid tissue. The cells are the so-called "principal" cells and they are embedded in a framework of reticular connective tissue. 15 μm.

general lymphoid hyperplasia. Hyperthyroidism is found in persons suffering from exopthalmic goitre or with toxic adenomas of the thyroid; there is a consequent increase in the basal metabolic rate. Enlargement of the thyroid without any increase of basal metabolism or overproduction of thyroxine is found in simple goitres due to iodine insufficiency and to nontoxic adenomas.

Decreased activity of the gland, on the other hand, is usually associated with a low flat epithelium (Fig. 15.8), and an excessive amount of colloid material which is deficient in

iodine content. If such hypothyroidism occurs in adults then the condition is known as *myxoedema*, a condition characterized by lethargy, slowing of the mental activities, a sallow appearance and puffy skin and dry, sparse hair. Malfunction of the thyroid in infancy gives rise to the condition known as *cretinism*, typified by a stunting of the growth and by mental retardation. In both myxoedema and cretinism the condition can be alleviated by the administration of thyroxine.

It must be remembered, however, that all parts of the gland do not exhibit the same degree of activity at any one moment: it is usual to find in sections that one microscopical field shows both active and resting follicles: and, further, in a single follicle there may be a segment of high, active epithelium while the remaining cells are of the low inactive type. The iodine content of the colloid material may vary very considerably and independently of the amount of the colloid itself.

(2) Heat and Cold

Exposure to cold stimulates the gland to activity: the mitochondria and Golgi apparatus are enlarged, the cells swell and the stored colloid is resorbed.

Heat induces inactivity of the gland, the mitochondria and Golgi apparatus shrink and the colloid accumulates.

PARATHYROID GLANDS (Fig. 15.9)

The parathyroid glands, usually four in number, are small rounded bodies found within the connective tissue capsule of the thyroid; very occasionally they may be embedded in the thyroid tissue. Aberrant parathyroids sometimes occur in the thymus.

The connective tissue capsule encloses densely packed groups of cells that are arranged in cords. The cells are of two kinds, non-granular *principal* cells and large *oxyphil* cells containing granules. The principal cells have a homogeneous cytoplasm which may stain faintly with acid dyes, and a vesicular nucleus: the granules of the oxyphil cells stain strongly with acid dyes (e.g. eosin), have small, deeply staining nuclei, and are not usually present before ten years of age. Between the cells is a framework of vascular reticular connective tissue in which mast cells and macrophages commonly occur. The principal cells contain glycogen.

The E/M shows that two functional states may be distinguished in the principal cells. The active state shows a cytoplasm rich in ribosomes and in rough surfaced endoplasmic reticulum and with a prominent Golgi apparatus. Electron-dense presecretory granules are plentiful in the cytoplasm, especially near to the borders of the cell which are close to blood capillaries. The inactive principal cells do not have much ER but are rich in glycogen; presecretory granules are absent. The oxyphil cells are characterized by the presence of numerous mitochondria which are rich in cristae and in a few glycogen granules squeezed in between the mitochondria. Other cell organelles are not apparent.

Blood Vessels and Lymphatics

The parathyroids have a very rich blood supply. The vessels enter at the hilum and the branches run in the septa, to break up into a network of anastomosing sinusoidal capillaries in the reticular connective tissue surrounding the cell-groups. The main vein also leaves at the hilum.

The blood vessels are accompanied by lymphatic vessels.

Nerves

The non-myelinated nerve fibres present are probably vasomotor.

Development

The parathyroids arise as solid evaginations from the epithelium of the third and fourth pharyngeal (branchial) pouches.

Function

The parathyroids are essential for life, as they are important for the regulation of the concentration of calcium ions in the body fluids. The active principal of the gland (*parathyroid hormone*) acts in response to a lowering of the blood calcium by stimulating the osteoclasts to resorb bone and free calcium ions from the reserve skeletal depots. The hormone also has an effect on the inorganic phosphorus concentration, an excess of the hormone causing the reduction of the plasma phosphate. This effect is most probably mediated by an action of the hormone on the kidney which causes the tubular reabsorption of phosphate to be inhibited, hence this ion is then lost in the urine.

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CHAPTER 16

THE PINEAL AND THE HYPOPHYSIS (PITUITARY GLAND)

THE PINEAL

The pineal body (epiphysis cerebri, conarium) (Fig. 16.1) is a small conical body attached by a short stalk to the roof of the third ventricle of the brain: the cavity of the ventricle is continued into the stalk as the pineal recess, lined by ependyma. The pineal body is covered (except where it is attached to the habenular and posterior commissures) with pia mater, which gives rise to connective tissue septa that carry many blood vessels into the organ. These septa separate the cellular elements into cords.

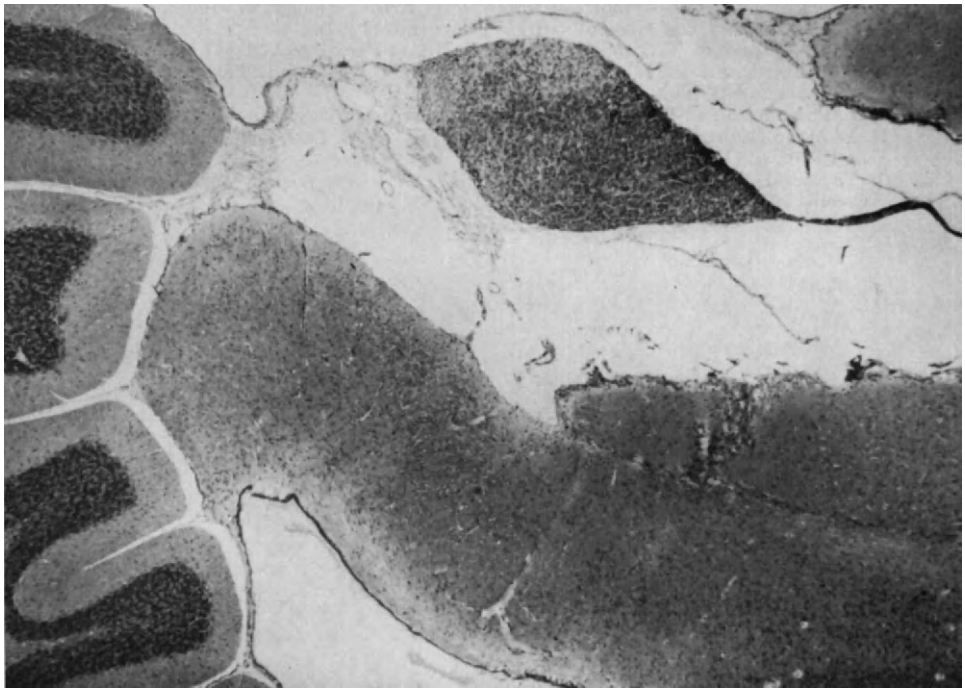


FIG. 16.1.—Low power micrograph of part of a sagittal section of a rat brain. The pineal may be seen at the top of the picture with the cavity of the third ventricle immediately beneath it. The cerebellum is to the left. Micrograph by Dr. H. M. Charlton. 250 μm.

The parenchyma of the organ is composed of cells termed *pinealocytes* or *chief cells*. These cells have large irregular nuclei and a pale cytoplasm; by specific silver impregnation techniques Hortega has shown that these cells have long processes with club-like endings apparently terminating in close proximity to the endothelia of the capillaries. The E/M shows the cytoplasm of these cells to be very rich in microtubules, with some rather atypical smooth surfaced endoplasmic reticulum and a moderate number of typical mitochondria. Lipochrome pigment droplets are often present.

Between the chief cells is found the *interstitial tissue*, characterized in the optical microscope by the elongated form of its nuclei. These cells are regarded by some authorities as atypical glial elements.

The pineal often shows the presence of extracellular concretions known as *corpora arenacea* (brain sand) (Fig. 16.2.)

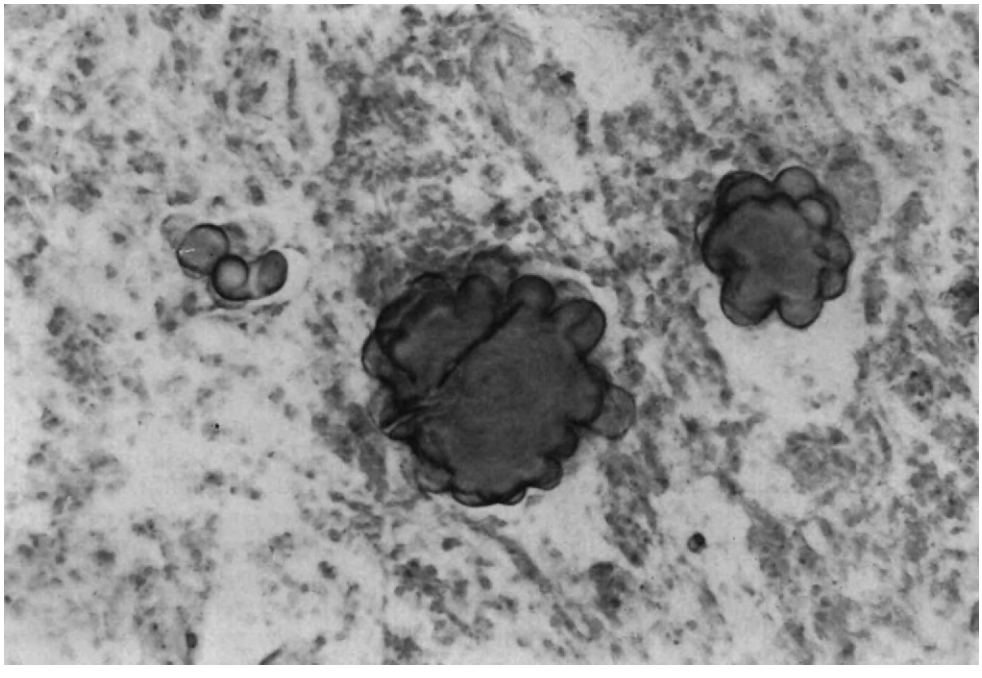


FIG. 16.2.—Micrograph of pineal tissue, with three accretions of "brain sand" (corpora arenacea) showing their typical lobulated form. 120 μm.

Function

The pineal seems to have a neuro-endocrine function and although much research is in progress at present, clear ideas on its exact role are still lacking. It seems, however, to participate in the regulation of the rhythmic activity of the endocrine system, possibly by the elaboration of specific hormones or compounds such as methoxyindoles. The prime stimulus for their secretion may well be mediated by visual reflexes.

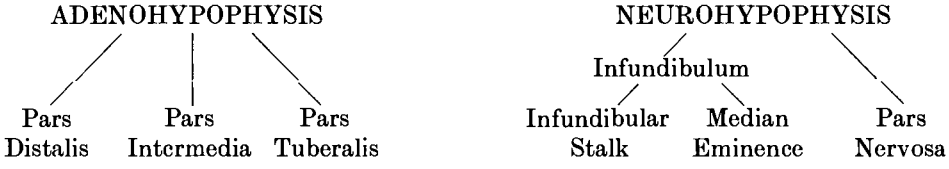
Changes with Age

The pineal body is said to increase in size up to seven years of age and then to undergo a very slow involution. Involution is characterized by a relative increase of interstitial tissue, and by hyaline changes in the septa and in the cells. "Brain sand" (*corpora arenacea*) increases as involution proceeds: it consists of laminated calcareous nodules (carbonates and phosphates of calcium and magnesium) that occur both in the pineal and in the various extensions of the pia mater.

THE HYPOPHYSIS (THE PITUITARY GLAND)

The hypophysis is located in the sella turcica of the sphenoid bone, and is attached by a stalk to the floor of the third ventricle. The hypophysis can be divided into two distinct parts (Figs. 16.3, 16.4); (*a*) the *adenohypophysis* which is a derivative of the buccal epithelium,

and (*b*) the *neurohypophysis*, which is derived from the floor of the diencephalon, i.e. neural ectoderm. The former may be subdivided into (1) the *pars distalis*, separated in the young by a cleft from (2) the *pars intermedia*. In the adult this cleft is represented by isolated vesicles and associated cells. The third subdivision is the *pars tuberalis*, associated with the tuber cinereum and forming part of the hypophyseal stalk. The neurohypophysis is itself divided into (1) *the median eminence*, (2) thc *infundibular stalk*, and (3) the *pars nervosa*. These subdivisions are summarized in the following table



The pars distalis was formerly referred to as the *anterior lobe*, the pars intermedia, together with the pars nervosa as the posterior lobe and the pars infundibularis, the infundibular stalk and the median eminence were sometimes loosely termed the *hypophyseal stalk*.

Development

The neurohypophysis is derived from the neural ectoderm, and the remainder of the gland from the buccal epithelium, a hollow outgrowth of which grows towards the developing brain as the pouch of Rathke. The advanced part of this outgrowth comes into contact with a hollow downgrowth from the floor of the third ventricle: this latter becomes the *pars nervosa*, while the part of the hollow buccal outgrowth in contact with it becomes the *pars intermedia*. The rest of the buccal outgrowth, separated from the pars intermedia by a cleft which is the remnant of the original lumen, develops into a large anterior portion which becomes the *pars distalis*, and two small lateral lobes which later fuse to form the *pars tuberalis*. The *pars tuberalis* then spreads round the stalk and beneath the tuber cinereum.

Normally, in man, all connection with the buccal epithelium is ultimately lost: the pars nervosa retains its connection with the floor of the third ventricle by a stalk, the infundibular stem, but the cavity in this neural portion is obliterated.

The part of the gland that is of buccal origin consists at first of tubules lined by cubical epithelium and united by vascular connective tissue: later the lumen of the tubules becomes obliterated and the cells are arranged as solid cords.

Pars Distalis

This is by far the largest part of the organ and consists of cords of epithelial cells supported by reticular fibres. Between the cords are sinusoids. Macrophages are associated with these spaces in the rabbit and probably, also, in the human.

The optical microscope shows epithelial cells of two kinds: (1) empty-looking chromophobes and (2) granule-containing chromophils. The latter may be further subdivided into (*a*) *acidophil cells* which contain granules stainable with acid dyes such as orange G and (*b*) *basiphil cells* whose granules stain magenta after the PAS technique (see p. 10) because of their content of glycoprotein; they may also be stained with aniline blue and other dyes. The appearance of these cells after a typical trichrome stain is shown in Plate 3 (facing p. 213).

The acidophil cells probably consist of two kinds; (1) mammotrophs, producing the hormone prolactin (LTH), and (2) *somatotrophs*, producing growth hormone (STH). The former usually contain very large secretion granules. The basiphil cells are also divisible into two types on the basis of their staining properties, shape, and ultrastructure; these are (1) *thyro-*

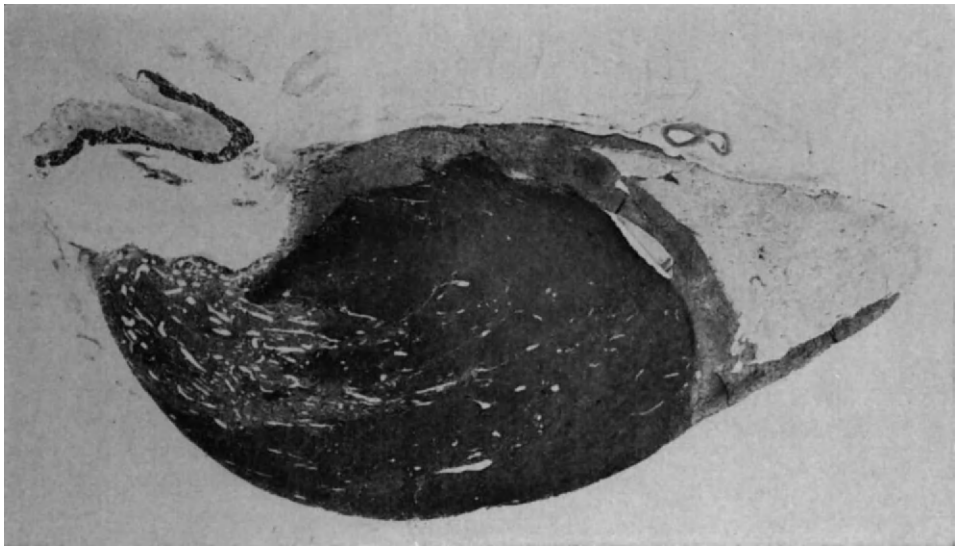


FIG. 16.3.—Sagittal section of a bovine pituitary, stained with haematoxylin and eosin. The blood vessels passing from the primary portal plexus to the pars distalis may be seen in the pars tuberalis on the left.

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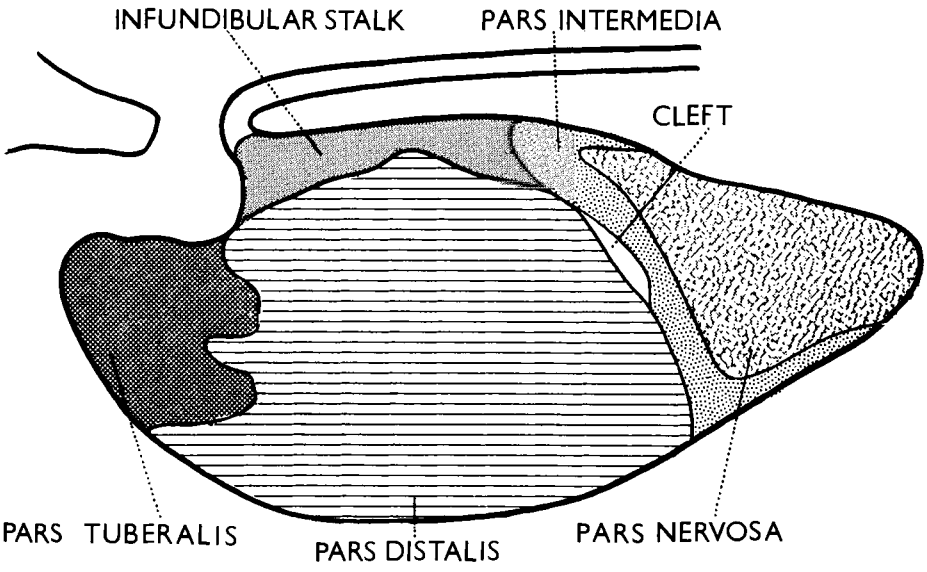


FIG. 16.4.—A diagram to illustrate the relationships of the parts shown in Fig. 16.3.

trophs, producing thyrotrophins (T.S.H.), and (2) *gonadotrophs*, producing gonadotrophins (follicle stimulating hormone, F.S.H., and luteinizing hormone, L.H.).

The granules of the different chromophil cells represent specific hormones or their precursors and a secretory cycle seems likely, during which there are alternating phases of granule synthesis and discharge. In the discharged condition, the cells would appear chromophobic under the optical microscope. The chromophobes were at one time thought to be cells which were undifferentiated and which could transform into the two types of chromophils. It now seems

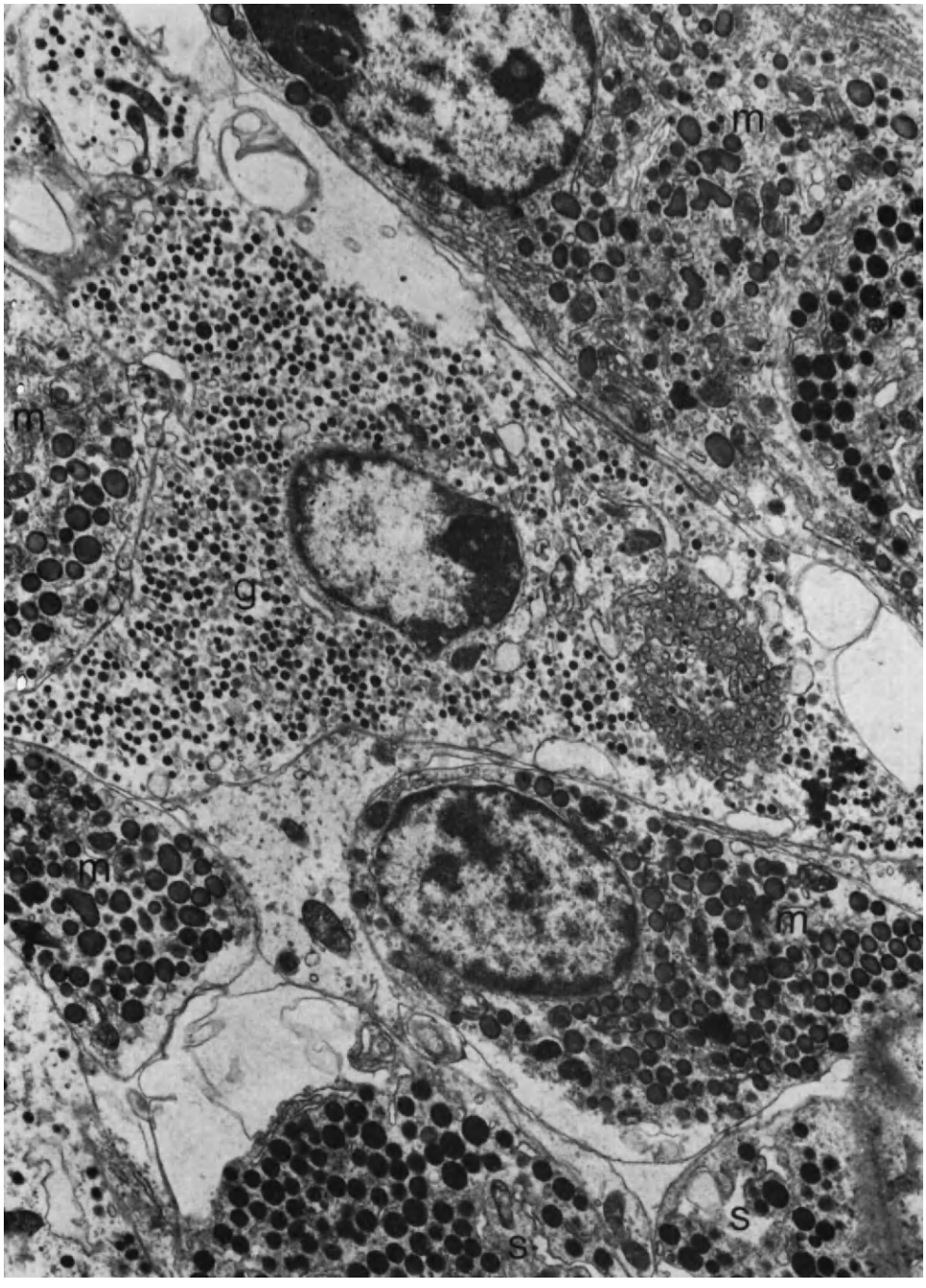
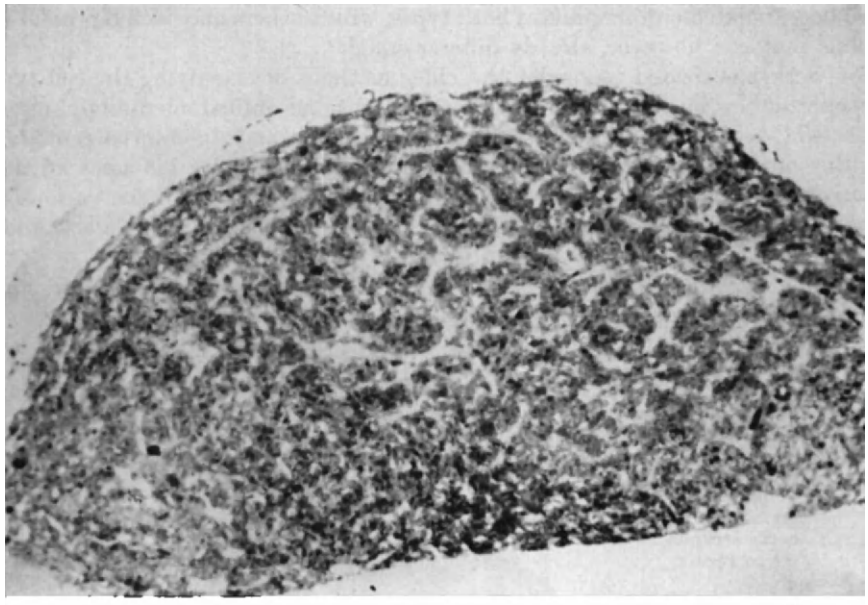


FIG. 16.5.—An E/M of part of the pars distalis of the vole. Three different cell types are visible; in the centre of the field is a gonadotroph with small granules (g). Parts of four mammotrophs (m) with large granules surround this cell, whilst two somatotrophs with large electron-dense granules (s) are at the bottom of the field. Preparation and micrograph by Dr. R. M. Worth.

1 μm.



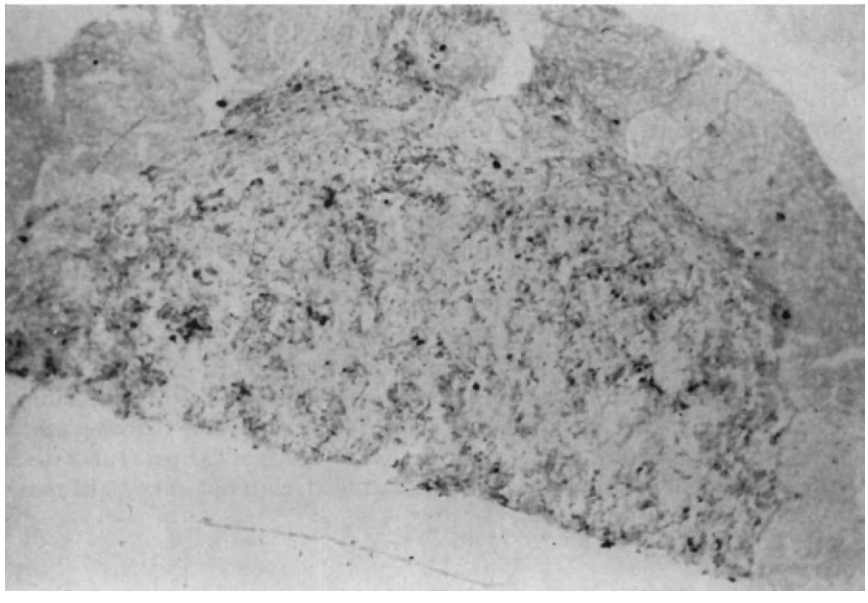


FIG. 16.6.—A, darkly stained neurosecretory material in the neurohypophysis of a vole; B, a similar preparation, but from an animal which had been subjected to dehydration. Note the disappearance of neurosecretory material. Micrographs from preparations by Dr. J. R. Clarke.

likely that the chromophobes are not a homogeneous population; some may well represent exhausted degranulated chromophils of both types, whilst others may well represent immature chromophils that are, however, already differentiated.

Recent work has tended to render the older methods of classifying the cell types in the adenohypophysis (by means of their staining reactions and optical microscopy) obsolete. The use of the E/M, together with homogenization of the cells and differential centrifugation to separate the various granules into discrete fractions, coupled with bio-assay of the physiological activity of each fraction has enabled accurate identification of the various cell types under the E/M to be made with certainty, largely on the basis of granule size and electron density (Fig. 16.5).

The granule sizes of the various cell types in the rat adenohypophysis are summarized in the following table (from McShan, 1971)

Cell type	Mean granule size (nm)	Range of granule size (nm)
mammotrophs	318	250–870
somatotrophs	240	100–390
gonadotroph (LH)	145	75–235
gonadotroph (FSH)	133	75–200
corticotrophs	106	50–180
thyrotrophs	85	40–145

E/M studies have also shown that the granules are released from the cells by the process of exocytosis.

Pars Intermedia

The pars intermedia is small in amount, ill-defined and much less vascular than the pars distalis. The cells are usually clear, but occasionally fine basiphil granules can be seen: they are often arranged around vesicles that contain a colloid material. The pars intermedia is sharply marked off from the pars nervosa, but merges into the pars distalis.

It is now known that melanocyte stimulating hormone (M.S.H.) is secreted by the pars intermedia. In lower animals this hormone seems to control the actual melanocyte expansion and contraction but in man where this does not occur, the hormone probably regulates melanin synthesis.

Pars Tuberalis

This part of the gland is extremely vascular and consists in man of solid cords of non-granular cells; these form a sleeve around the stalk. The high degree of vascularity is largely due to the fact that this part of the gland carries the hypophyseal venous portal system en route from the median eminence to the pars distalis. The cells of the pars tuberalis sometimes surround a few irregular cavities that contain a colloid material. They are not known to secrete any hormone.

The Neurohypophysis

The essential functional components of the neurohypophysis are groups of axons of neurosecretory cells whose cell bodies are located in certain nuclei of the hypothalamus. The axons pass down through the infundibular stalk to end in the substance of the pars nervosa. Both in the neurons, and in their axons, granular neurosecretory material is present and this stains characteristically with certain dyes (Fig. 16.6). The available evidence suggests that these neurosecretory granules are either a precursor or a carrier of the two characteristic hormones of the infundibular process: *oxytocin* and *antidiuretic hormone* (ADH). Thus, although

the hormones accumulate in the pars nervosa they are probably formed in the neurons situated in the supra-optic and paraventricular nuclei of the hypothalamus.

In addition to axons, the infundibular stalk contains blood vessels and connective tissue. In general, however, none of these elements is arranged in a particularly orderly way, but in the opossum the pars nervosa shows a lobulated structure and a close association between axon terminals and small blood vessels is easily demonstrable. Presumably a similar close relation occurs in other species, including man, whereby the neurosecretory material leaves

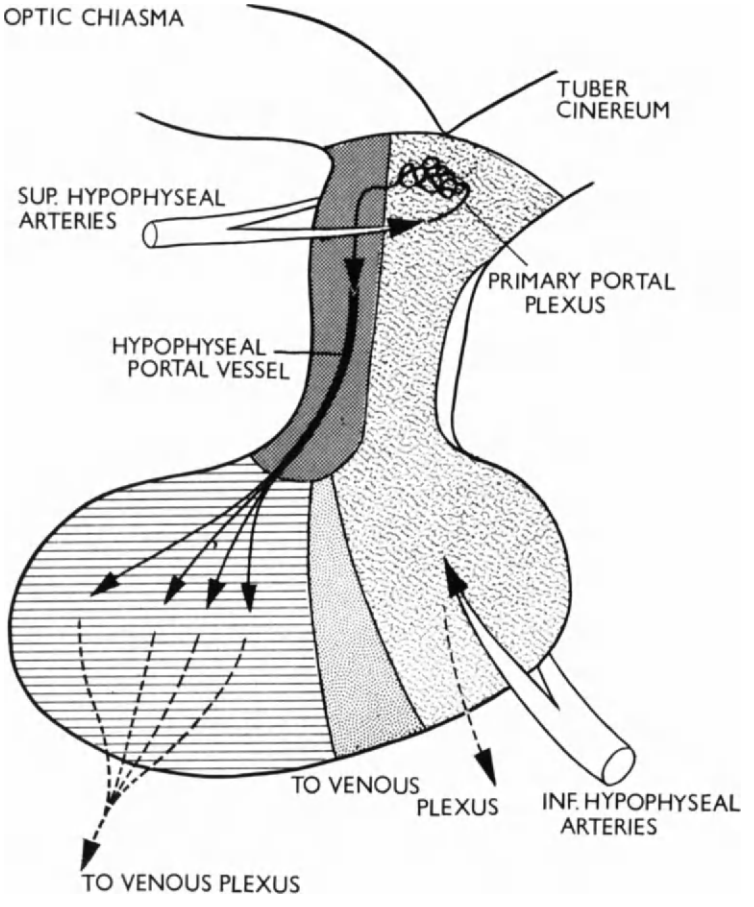


FIG. 16.7.—A diagram of the blood supply of the hypophysis.

the axon terminals to pass into the circulation. Large stainable amorphous bodies (known as *Herring bodies*) represent accumulations of neurosecretion in the axoplasm of the fibres of the hypothalamo-hypophyseal tract.

Finally, there are neuroglia-like cells called *pituicytes*. These are cells with branching processes and cytoplasmic granules which blacken with osmium tetroxide. They are probably supporting, rather than secretory cells as was formerly believed.

Basiphil cells are often found and these have presumably migrated in from the pars distalis.

Blood Vessels (Fig. 16.7)

The paired superior and inferior hypophyseal arteries together supply the hypophysis with blood. The superior arteries enter the hypophyseal stalk and break up into a capillary plexus

which forms the hypophyseal portal vessels; these course down through the pars tuberalis on the anterior and lateral aspects of the hypophyseal stalk. At the point of junction between the pars tuberalis and the pars distalis they spread out and break up into sinusoids which ramify between the glandular cells of the pars distalis.

The endothelial cells of the primary capillary plexus are in close relation with the terminations of the nerve fibres of the hypothalamo-hypophyseal tract (Fig. 16.8) and it appears that the pars distalis is under hypothalamic control through the mediation of a neuro-vascular link. The pars nervosa receives a direct and independent blood supply from the ramifications of

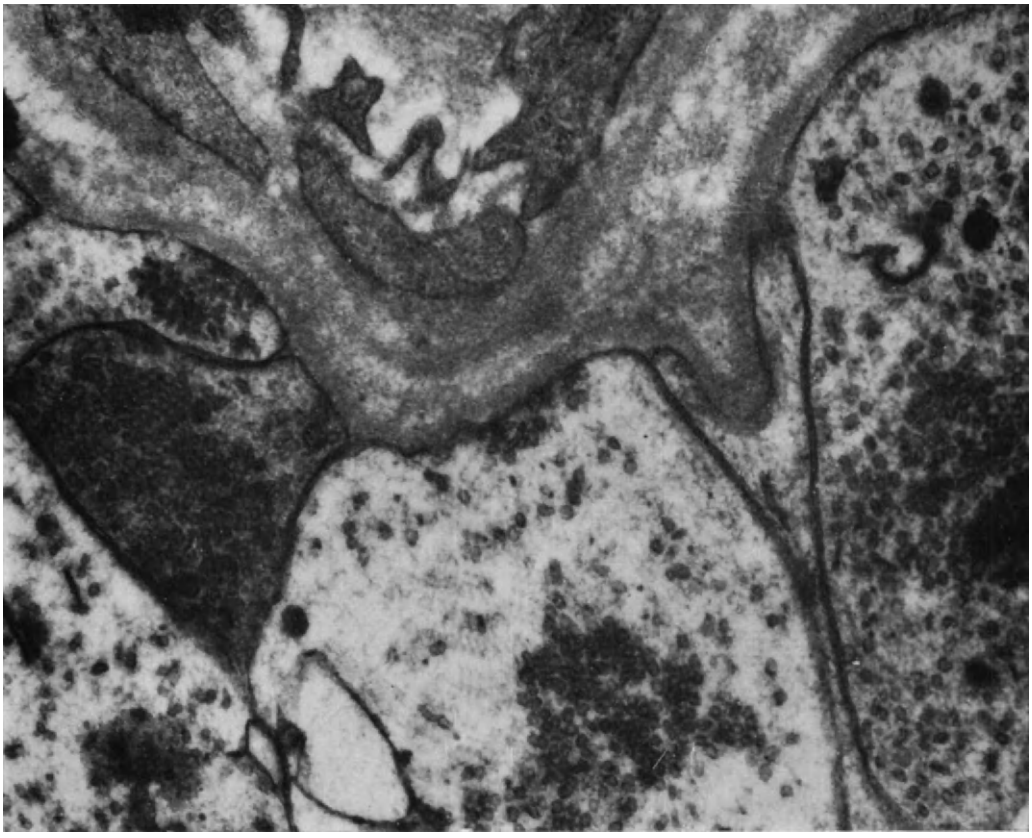


FIG. 16.8.—An electron micrograph of nerve terminations in the median eminence to show their close relationship to a capillary (at the top of the micrograph); the nerve terminals contain numerous synaptic vesicles. 0·2 μm.

the inferior hypophyseal arteries, whilst the median eminence and the infundibular stalk have an arterial supply from the primary portal vessels.

No lymphatic vessels have been described.

Nerves

The pars distalis receives post-ganglionic sympathetic fibres from the carotid plexus. The pars nervosa receives fibres from the supra-optic hypothalamic nuclei, which end in relation to the blood vessels: a few fibres from nerve cells in this region may also supply the pars distalis and pars intermedia. Any nerve fibres passing to the pars distalis are believed to be concerned with its vascular components; there is no evidence for any direct innervation of the glandular cells.

Variations in Structure under Different Conditions

(1) Age

In the young a well-marked cleft between the pars distalis and the pars intermedia is present: in the adult this tends to be replaced by isolated cysts or to disappear altogether. Occasionally, in young infants, the posterior lobe contains typical racemose glands secreting a colloid material into the cleft.

(2) Other Conditions

A great increase in growth is usually associated with an increase in the pars distalis, due to a great increase of the acidophil cells. If the hyperpituitarism develops before the union of the epiphyses of the long bones *gigantism* results, while if it occurs after this union, the bony overgrowth is limited to the lower half of the face, the hands and the feet, and the condition is known as *acromegaly*.

For details of the actions of the various hormones produced by the hypophysis a textbook of endocrinology should be consulted.

References for further detailed study:

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CHAPTER 17

THE SKIN

The whole surface of the body is covered by the skin which, together with its specializations (nails, hair), forms a protective, elastic, waterproof and sensitive covering. This consists always of two parts, an *epidermis* of ectodermal origin and an underlying *dermis* derived from the mesoderm. Beneath the dermis is the subcutaneous layer of loose connective tissue connecting the skin with the subcutaneous fascia: in some parts this subcutaneous tissue is modified into fatty connective tissue, the *panniculus adiposus*. The epidermis is sharply marked off from the dermis, but the latter blends with the subcutaneous tissue, and with superficial muscle where present (e.g. platysma muscle).

Macroscopic appearance of the Skin

Clear differences may be seen in the appearance of the skin over the various parts of the body; hairs may be present in abundance or in only very small amounts or they may be absent altogether (as in the sole of the foot and in the palm of the hand) and there may be pronounced local differences in its colour and texture which reflect variations in the distribution of glands and underlying blood supplies.

Folds or creases may be seen; these fall into two categories:

(1) Major creases, where the skin passes over an underlying joint in the bony skeleton;

(2) Minute furrows which intersect and bound small angular spaces. These may be seen to advantage on the dorsum of the hand.

In the region of thick hairless skin on the palm of the hands and the tips of the fingers prominent ridges may be seen (Fig. 17.1). These are arranged in very definite patterns which are so unique that the fingerprint may be used to identify any individual with absolute certainty. Fig. 17.1 also shows clearly the very minute pores on the surface of the ridges which represent the openings of the sweat glands.

The colour of the skin is basically due to three separate features:

(1) The intrinsic yellowish colour of the keratinized epithelium.

(2) The pigment, usually melanin, which is elaborated in the melanocytes and is probably transferred from them to other cells in the epidermis. Such intrinsic pigmentation varies very much between races and individuals and between different areas of the body.

(3) The red colour of the blood pigments shows through and any change in the colour of these may be reflected in the surface of the skin; such changes are of value in diagnosis.

Epidermis (Figs. 17.2, 17.3)

The epidermis consists of stratified keratinized epithelium. Two layers can always be distinguished: (1) the superficial keratinized layer, known as the *stratum corneum*, and (2) the deeper soft layer immediately covering the dermal papillae and known as the *stratum germinativum*. Between these two layers, particularly in parts where the skin is thick, three additional layers are usually differentiated, a *stratum lucidum*, a *stratum granulosum* and a *stratum spinosum*. The deepest cells of the stratum germinativum are columnar and frequently show mitoses. Mitoses, however, are not restricted to this layer and it seems likely that the *s. spinosum* as a whole is capable of mitotic activity, although some zones may be more active than others. The cells above this basal layer are polyhedral and there are minute spaces between them that are occupied by intercellular matrix. The surface of these polyhedral cells is apparently covered with minute processes which contact similar processes of adjacent

FIG. 17.1.—A photograph of the skin on the finger tip. The ridges which form the pattern of the finger print may be clearly seen, together with the pores on the surface of the ridges which represent the openings of the sweat glands.

1 mm.

cells thus forming bridges linked by desmosomes across the intercellular spaces: these cells are consequently known as "Prickle cells" (Fig. 17.4). The cytoplasm contains minute fibrils in parallel bundles which converge on the desmosomes and terminate in them. The superficial layer of the stratum spinosum is covered by the stratum granulosum, consisting of one to five layers of flattened polyhedral cells containing deeply staining granules consisting of keratohyalin. External to the stratum granulosum is the stratum lucidum, consisting of clear flat cells with boundaries so indistinct that the layer appears homogeneous. These cells contain eleidin, which appears to be derived by a transformation of keratohyalin. The stratum corneum consists of layers of dead, flattened cells, the most external of which are like scales

and continually being flaked off; these result from a final transformation of keratohyalin into the protein keratin. The lost cells are replaced by pushing up of cells from the underlying Malpighian layer. The outer layers are heavily keratinized, thus affording mechanical protection to the structures below and preventing them from drying. In the thin skin covering most of the body the stratum lucidum and the stratum granulosum cannot be distinguished: these layers are best developed in the thick epidermis of the palms and the soles, in which the stratum corneum may reach a thickness of over a millimetre (Fig. 17.3).

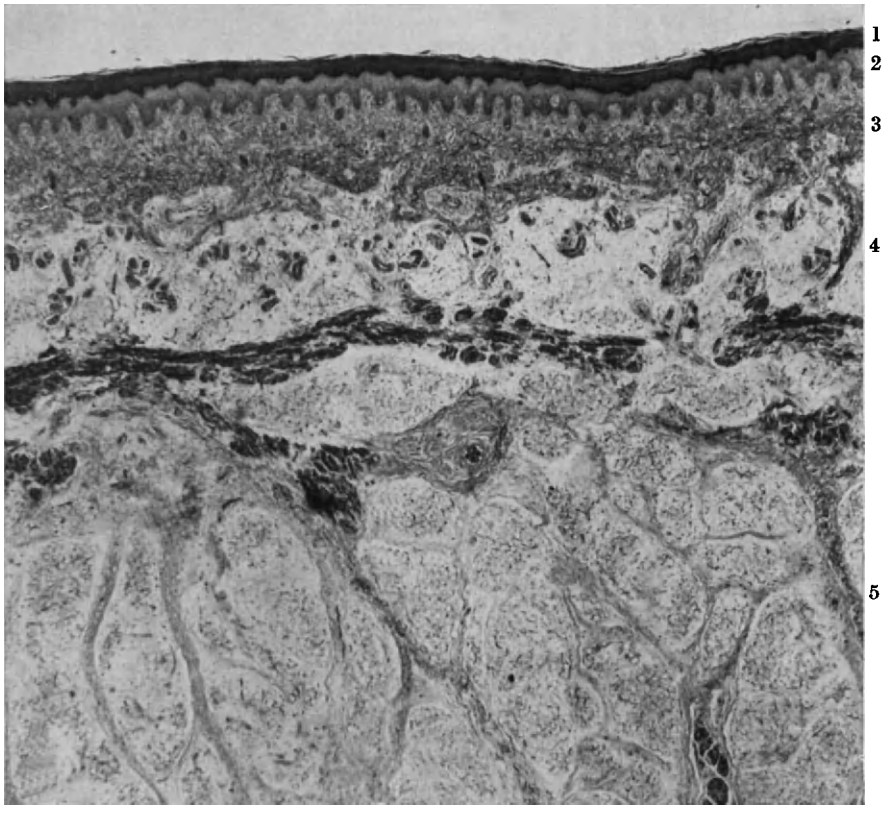


FIG. 17.2.—A section through the skin of the palm of the hand. 1, Stratum corneum; 2, Stratum germinativum with the dermal papillae; 3, dermis; 4, deep layer of the dermis containing the sweat glands; 5, subcutaneous adipose tissue. 500 μm.

The colour of the skin in pigmented races depends on the presence of melanin granules in the deepest cells of the stratum spinosum. In negroes all the epidermal layers contain pigment.

At the junction of the epidermis with the dermis (see below) there occur numbers of melanin-containing branching dendritic cells or melanoblasts derived, it is thought, from neural crest tissue. These send processes in among the cells of the str. germinativum and there is reason to believe that the melanin present in the deeper cells of the str. germinativum is produced by these melanoblasts, but exactly how the transference of the melanin is achieved is by no means clear. Melanoblasts contain the enzyme *dopa oxidase* which is involved in the normal production of melanin from a substrate, itself derived from tyrosine. Incubation of slices of fresh tissue with appropriately buffered *dopa* (dihydroxyphenylalanine) results in the formation of *dopa melanin*, which, unlike the golden brown of true melanin, is black in colour.

Other dendritic cells (Langerhans cells) can be demonstrated in the epidermis by appropriate techniques (Fig. 17.5). It was at one time thought that the Langerhans cells were effete melanocytes, but they have been shown to be active in the uptake of tritiated thymidine and their E/M appearance suggests also that they are active cells. Their function is still unknown.

Dermis (Fig. 17.2)

The dermis consists of connective tissue which is very dense where it adjoins the epidermis and becomes more open and loose in the deeper part where it becomes continued into the

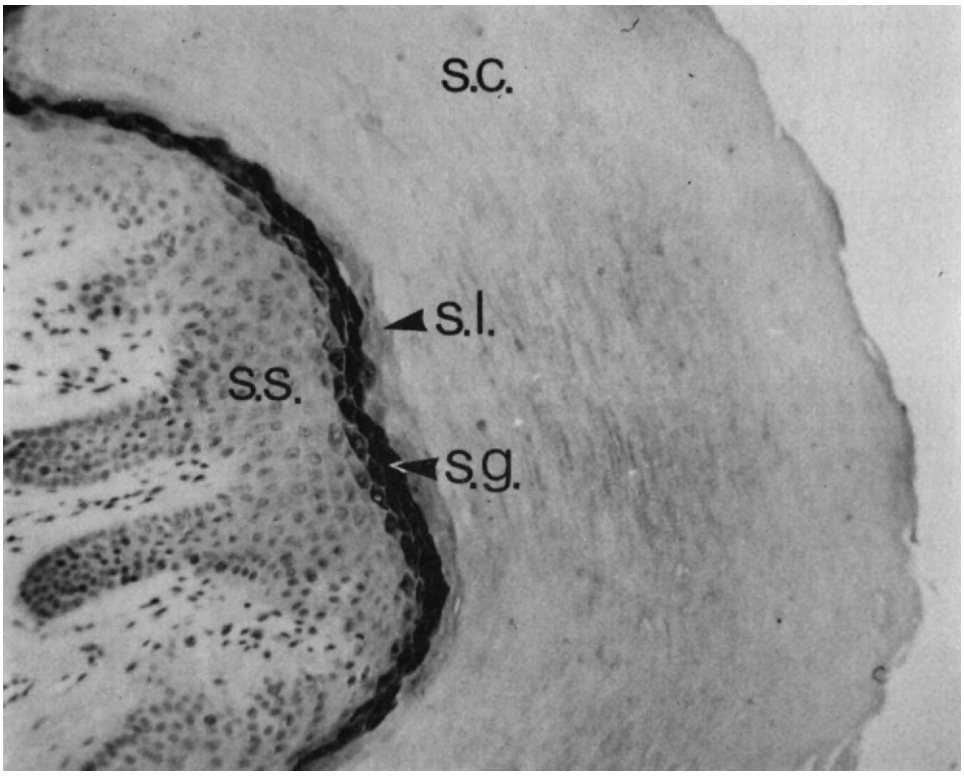


FIG. 17.3.—A micrograph of the epidermis (at a higher power). The thick stratum corneum is clearly shown at the top. The darkly staining cells of the stratum granulosum cross the middle of the picture, lying superficial to the cells of the stratum spinosum and the stratum germinativum. The collagen fibres of the dermis may be seen at the bottom of the photograph. 100 μm.

loose subcutaneous tissue. It varies greatly in thickness, and is usually thicker on the dorsal surface and in men than it is on the ventral surface and in women.

The surface of the dermis is covered with minute papillae or ridges which project into the epidermis. These papillae are very vascular and contain also a plexus of fine elastic fibres and many nerve endings. Beneath the papillae the connective tissue becomes very dense, the collagen bundles running in every direction: this arrangement, together with the presence of networks of thick elastic fibres between the collagen bundles, gives the skin its elasticity. The bundles that lie parallel with the lines of tension of the skin are particularly numerous and well-developed: an incision parallel with such a line gapes much less than one made across it, and such a wound therefore heals with little scar tissue.

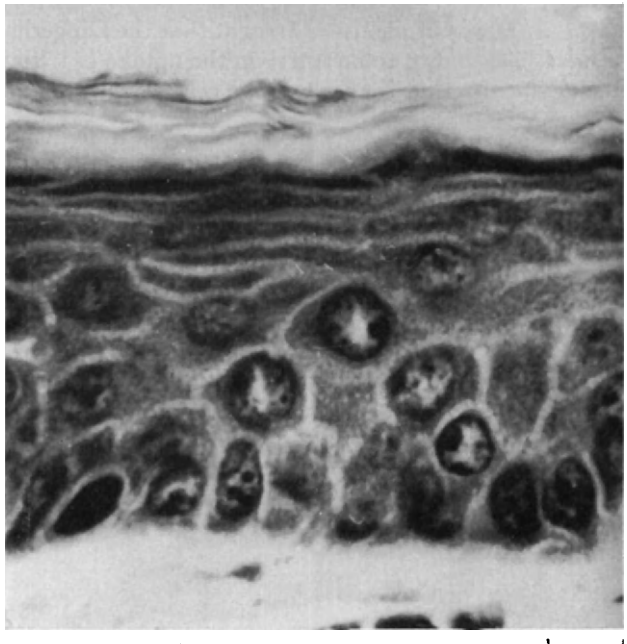


FIG. 17.4.—A micrograph of the skin from the forearm. The cells of the stratum spinosum are very prominent here; the separation between the adjacent cells is largely artificial. Note the thinness of the stratum corneum in this region.

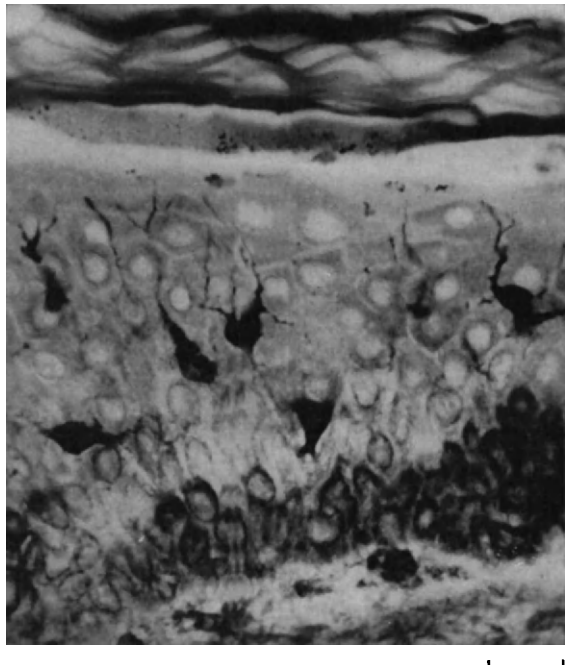


FIG. 17.5.—Langerhans cells (darkly staining) in the skin. (A. Breathnach, *Brit. J. Dermat.*, 1962.)

Smooth muscle fibres are present in the deeper parts of the dermis in the penis, the scrotum and the nipple, and also in relation to the hair follicles and to the sweat glands. Striated muscle may reach into the dermis in the skin of the face and of the neck.

The sweat glands (p. 248), sebaceous glands (p. 254), and hair follicles (p. 252) are found at various levels in the dermis.

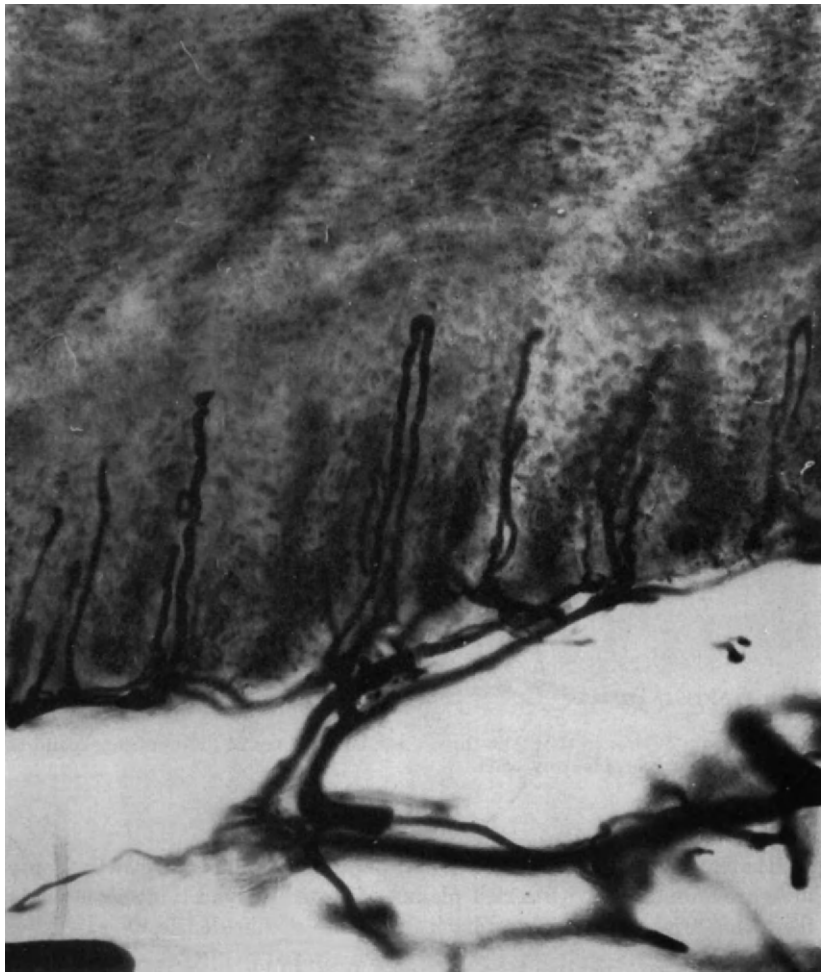


FIG. 17.6.—A micrograph of a thick section of skin in which the blood vessels have been injected. The epidermis is at the top of the picture. 30 μm. Note how the capillaries from the superficial plexus in the dermis loop between the dermal papillae.

Blood Vessels (Figs. 17.6, 17.7)

Large arteries at the level of the deep fascia provide a horizontal vascular plexus from which arise branches to supply the adipose tissue and other structures in the superficial fascia (e.g. sweat glands). A second horizontal plexus arises just below the dermis and from this capillaries arise to supply structures in this layer, especially the hair follicles and the papillae of the dermis. A third dense capillary plexus lying superficially in the sub-papillary region of the dermis sends capillary networks within every papilla. The venous drainage is by a parallel

series of plexuses at various levels, emptying ultimately into large subcutaneous veins. A special type of arteriovenous anastomosis, the glomus (see p. 189), is found in the skin, particularly in the pads of the fingers and of the toes and beneath the nails: it is concerned with temperature regulation.

The hair follicles, sebaceous glands and sweat glands all have an abundant blood supply. No blood vessels penetrate into the epidermis.

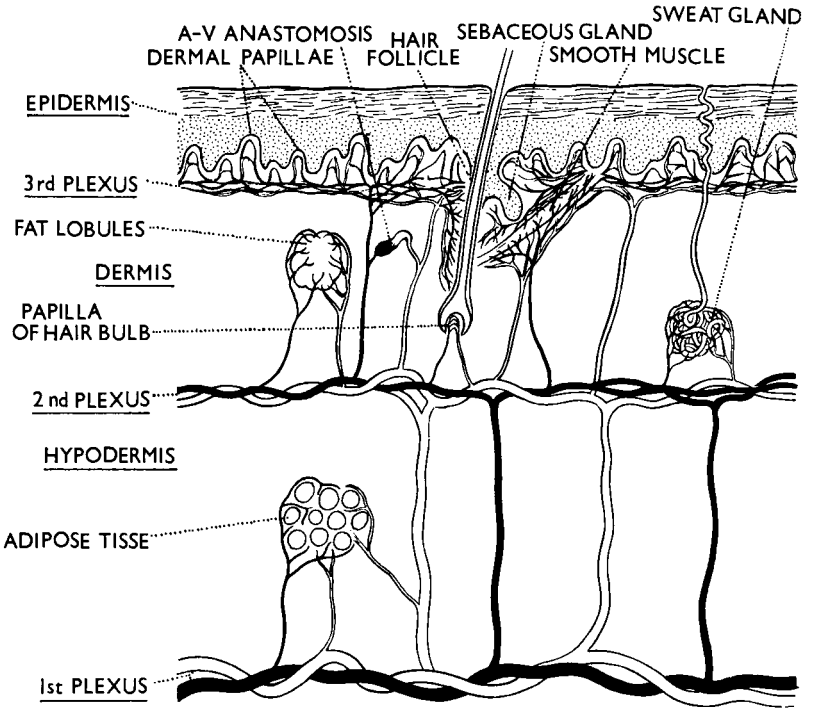


FIG. 17.7.—A diagram of skin to show the three vascular plexuses at different levels and the main structures which are supplied by each.

Lymphatics

The lymphatic capillaries begin in the dermal papillae as blind outgrowths joining to form networks. These are connected with a rich plexus of larger, valved lymphatic vessels beneath the dermis: from this arise the large lymphatics that run alongside the blood vessels.

Nerves

The skin is very richly supplied with nerve fibres, both sensory and of the sympathetic system. Vasomotor nerves supply the blood vessels, secretomotor nerves pass to the sweat glands, and pilomotor fibres to the hairs. But the most important fibres are afferent in function, the skin serving to collect sensory information from the exterior. The thick nervous networks of the subcutaneous layer provide various fine plexuses at different levels; of these the sub-epithelial and sub-papillary networks are the most clearly marked. Various types of nerve ending are found, of which the corpuscles of Meissner in the papillae and the Pacinian corpuscles in the deep layers are most easily identified (see p. 159). The sub-epithelial plexus provides fine fibres that penetrate between the cells of the epidermis, often ending in small varicosities. The hair follicles are supplied by basket-like nets (Fig. 17.14) and free nerve endings.

The appendages of the skin include the nails, the sweat glands, the hairs, the sebaceous glands, and the mammary glands: the last-named are described with the organs of reproduction (p. 390).

Functions of the Skin

The skin, which forms about 15 per cent of the total bodyweight, is of great importance as the barrier between the external environment and the body tissues. The functions of the skin may be listed as follows:

(1) Mechanical protection of the underlying tissues from mechanical trauma. The keratinization of the superficial layers of the epidermis is of great significance in this respect.
(2) Protection of the underlying body tissues against water loss; again this is a function of the keratinization. The impervious nature of the keratin must not be overstressed, however, as there is always some "insensible perspiration", i.e. water loss through the keratin, and it is easy for some substances, especially chemical poisons and insecticides to penetrate this barrier.
(3) Protection of the body tissues from the entrance of micro-organisms.
(4) Reception of tactile, thermal and other sensory stimuli; this is reflected in the extremely rich nervous supply of the skin.
(5) Thermoregulation of the body; the important role of the skin in this process is consequent upon the presence of the arterio-venous glomus (see p. 189) and a rich capillary network of vessels and upon the secretion of sweat which cools the body surface by its latent heat of evaporation.
(6) Accessory role in excretion; this is again mediated by the loss of various substances in the sweat.

THE NAILS (Figs. 17.8, 17.9)

The nail consists of modified epidermis. It is made up of:

(*a*) a *free edge*, the unattached extension;
(*b*) a *body*, the attached and visible uncovered portion;
(*c*) a *root*, the part lying beneath the skin.

The part of the skin upon which the nail lies is the *nail bed* or matrix: the nail bed is bounded on each side by folds of skin, the *nail wall*, and between the nail wall and the nail bed is a furrow, the *nail groove*.

The free edge of the nail gradually wears off or is cut off. The body of the nail is for the most part pink, due to the underlying vascular tissue, but near the root it becomes white, this part being called the *lunula*.

The substance of the nail is hard and horny, and is developed from the stratum lucidum: it is homologous to the stratum corneum consisting of layers of flat, clear, nucleated, cornified cells. This horny layer rests upon a typical epidermis, the stratum granulosum of which is modified to contain special onychogenic granules. The dermis underlying the nail bed is covered with longitudinal ridges instead of the usual irregular papillae: these ridges are very vascular, so that the overlying transparent nail looks pink. At the junction of the nail and skin in the nail groove the stratum corneum extends over the nail as the *eponychium*. At the place where the nail becomes free from its bed there is a similar extension beneath the nail known as the *hyponychium*. The nail grows from that part of the nail bed that appears whitish, namely, the lunula: as growth proceeds the nail is pushed forward.

The nail bed is richly innervated. Many of the fibres ramify in the dermal ridges, some extending into the epidermal layers: more deeply there are many Pacinian corpuscles.

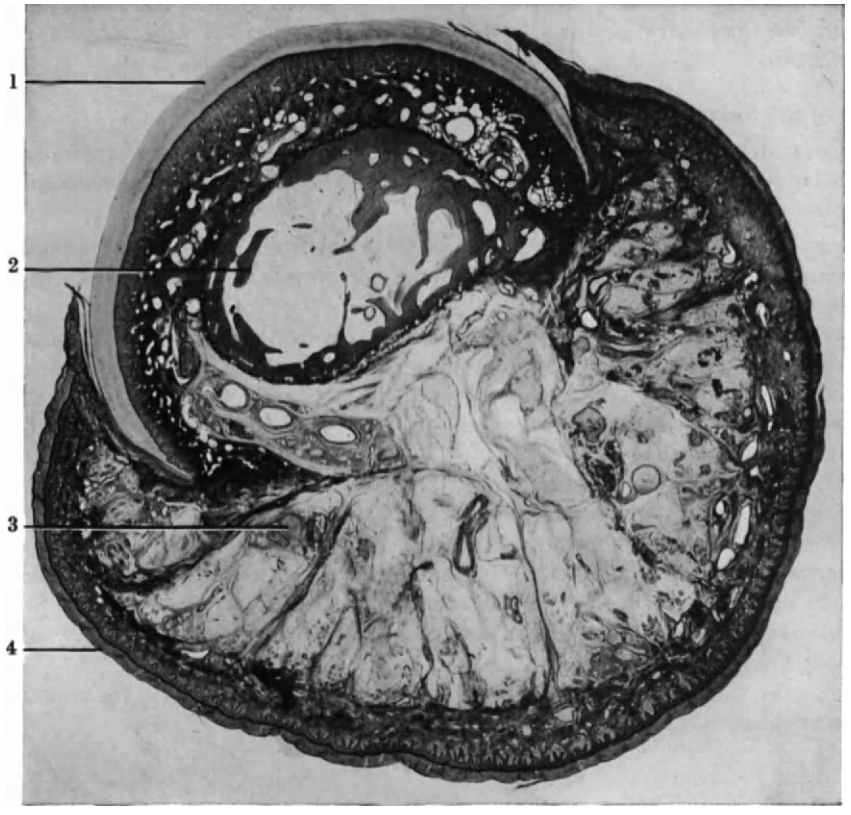


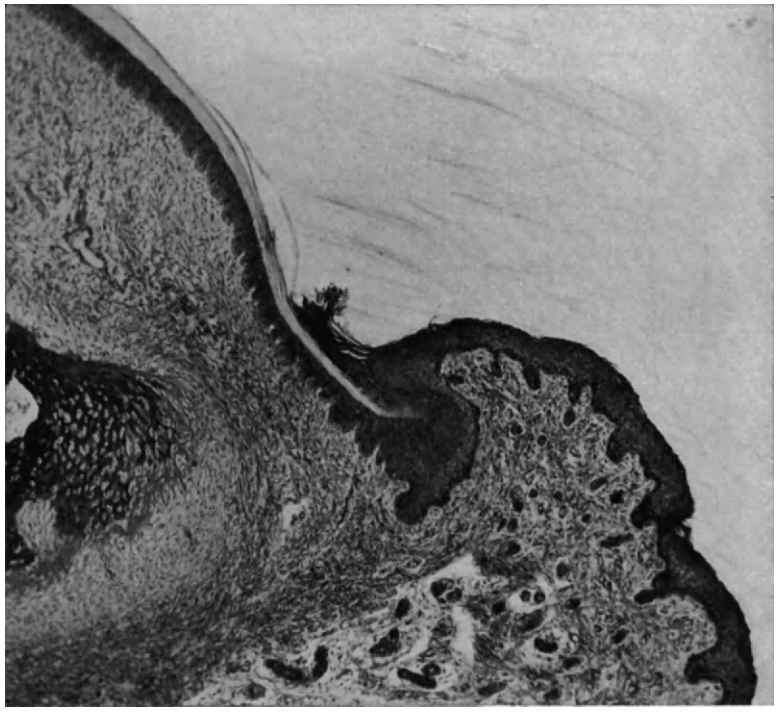
FIG. 17.8.—Cross section of the finger tip of an adult. 1, nail; 2, bone of the terminal phalanx; 3, Pacinian corpuscle; 4, skin. 0·25 cm.

THE SWEAT GLANDS

The sweat glands are found all over the body except at the margin of the lips, in the nail bed, the prepuce and the glans penis: they are particularly abundant in the skin of the palm of the hand and of the sole of the foot.

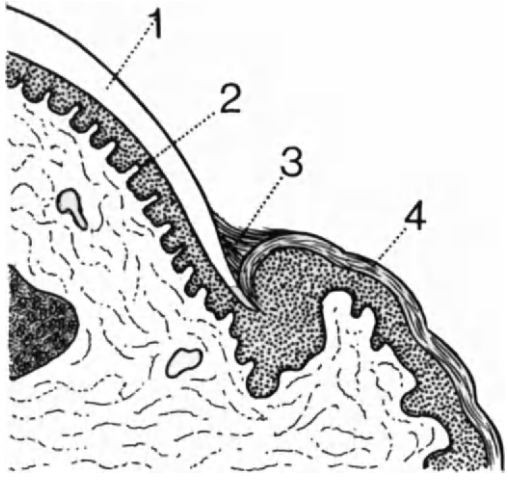
The typical sweat gland (*eccrine* or *merocrine* type) consists of a coiled tube lying deeply in the dermis (Fig. 17.10): the duct runs straight upwards through the dermis to reach the epidermis between two papillae: it then becomes coiled as it passes through the epidermis and finally opens on the surface as a sweat pore.

The tube constituting the secretory part of gland is lined by a simple, cubical epithelium (Fig. 17.11): in small glands this rests directly on a basal lamina, in larger glands there is a layer of flattened, spindle-shaped cells between the two, the myo-epithelial cells. The duct as it passes through the dermis is lined by two layers of cubical epithelium with a basal lamina; in its passage through the lower epidermal layers it is lined by cells of this zone arranged concentrically and when it reaches the stratum corneum the cellular wall is lost and the duct becomes merely a passage excavated through this layer to the surface. The blood vessels make a capillary network outside the basal lamina. The glands are supplied with many sympathetic non-myelinated nerve fibres, which make a plexus outside the basal lamella: from this arise cholinergic fibres which penetrate to ramify as varicose threads in relation to the myoepithelial cells and secreting cells.



A

300 μm.



B

FIG. 17.9.—A, longitudinal section of finger tip through the nail fold, full term foetus. B, diagram of the structures shown in Fig. 17.9A. 1, nail; 2, germinative layer covering the dermal ridges of the nail bed; 3, eponychium; 4, skin.

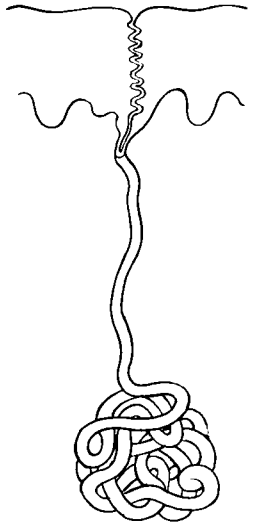


FIG. 17.10.—A diagram of an eccrine sweat gland. The coiled secretory part lies deep in the dermis; note the relatively straight course of the duct through the dermis and its spiral course through the epidermis.

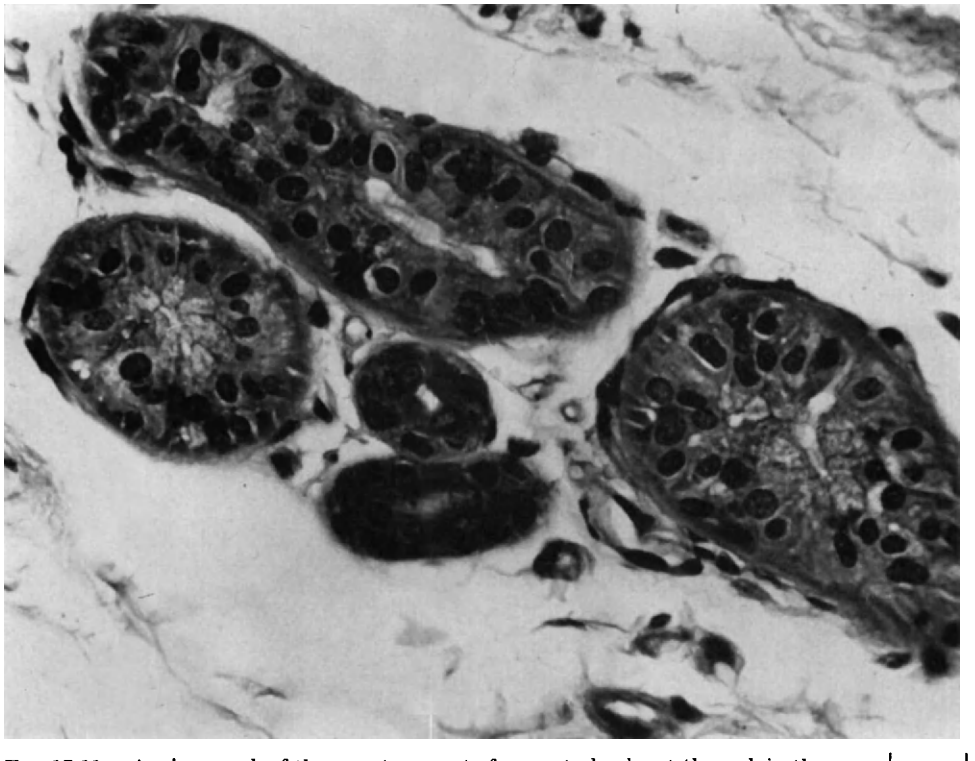


FIG. 17.11.—A micrograph of the secretory part of a sweat gland, cut through in three places. Below there are two sections through the more darkly staining cells of the first part of the duct.

15 μm.

Specialized Sweat Glands

(1) **Ceruminous glands** in the external auditory meatus.

These are large glands with branching secretory portions containing many large myo-epithelial cells. The glandular cells contain large pigment granules containing lipid. The ducts sometimes open with those of the sebaceous glands into the sheaths of the hairs. The secretion is fatty, not watery.

(2) **Apocrine glands** in the axilla, round the anus, on the labia majorae and the scrotum.

These are very large branched tubular glands which produce their secretion by disintegration of the free ends of the cells that line the alveolus. This method of secretion is similar to that of the mammary glands. Some authors maintain that they are innervated by adrenergic nerves, whilst others believe that secretion of the apocrine glands is independent of any nervous control.

The apocrine secretion is scanty and viscous; originally odourless, it is rapidly broken down by bacteria to give characteristic body odours.

(3) **Ciliary glands (of Moll)** in the eyelid.

These are modified sweat glands in which the terminal part is not coiled into a ball but contains a large lumen. The ducts may open into the sheaths of the eyelashes.

THE HAIRS

The hairs are developed from the epidermis and are present all over the body except on the palms, the soles, the lateral surfaces and backs of the terminal phalanges of the fingers and of the toes, the lips and parts of the external genitalia.

The hair consists of a *shaft* projecting above the surface of the skin and a *root* which is the part within the skin. At its deep end the root expands into a bulb, the *hair bulb*, which is

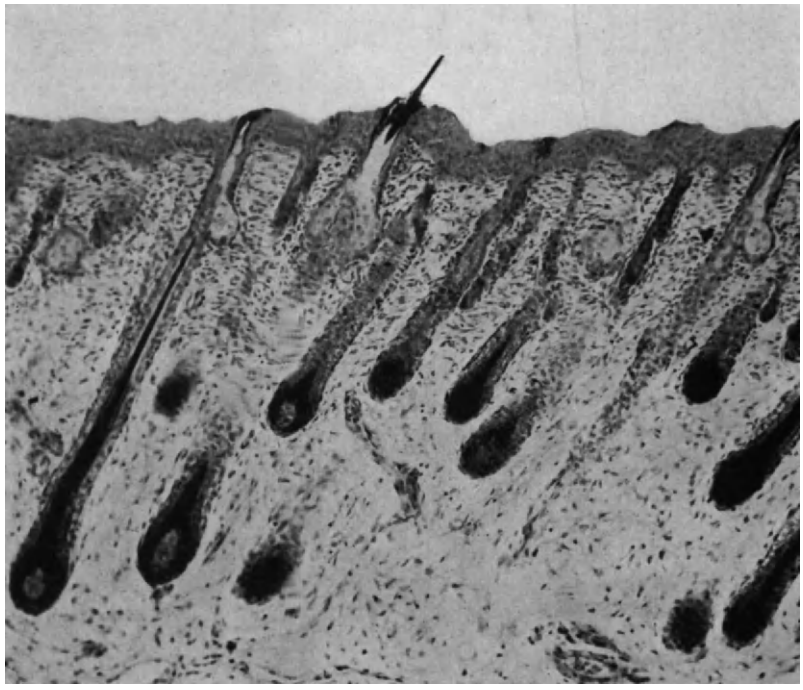


FIG. 17.12.—A vertical section of the scalp to show the typical appearance of hairy skin. Many hair follicles are visible. 300 μm.

moulded over a specialized vascular portion of the dermis called the *hair papilla*. The *hair follicle* (Fig. 17.12) encloses the root of the hair. The hairs are set at an acute angle in the skin and do not project vertically: the slope varies in different parts, giving rise to definite patterns. *Sebaceous glands* are connected with the hairs and these open into the upper part of the follicle.

Structure of the Hair

The main substance of the hair consists of horny fibrous protein (*keratin*), which can be separated by acid into long spindle-shaped cornified cells that show longitudinal fibrils. In coloured hair, pigment is present between these cells. The hair is covered by a very thin membrane (*cuticle*) that consists of transparent, cornified epithelial cells, arranged like the tiles of a roof with the free edge directed to the surface. In the thick hairs the centre is occupied by a *medulla* of irregular cornified cells. Minute air bubbles are often present in both cortex and medulla.

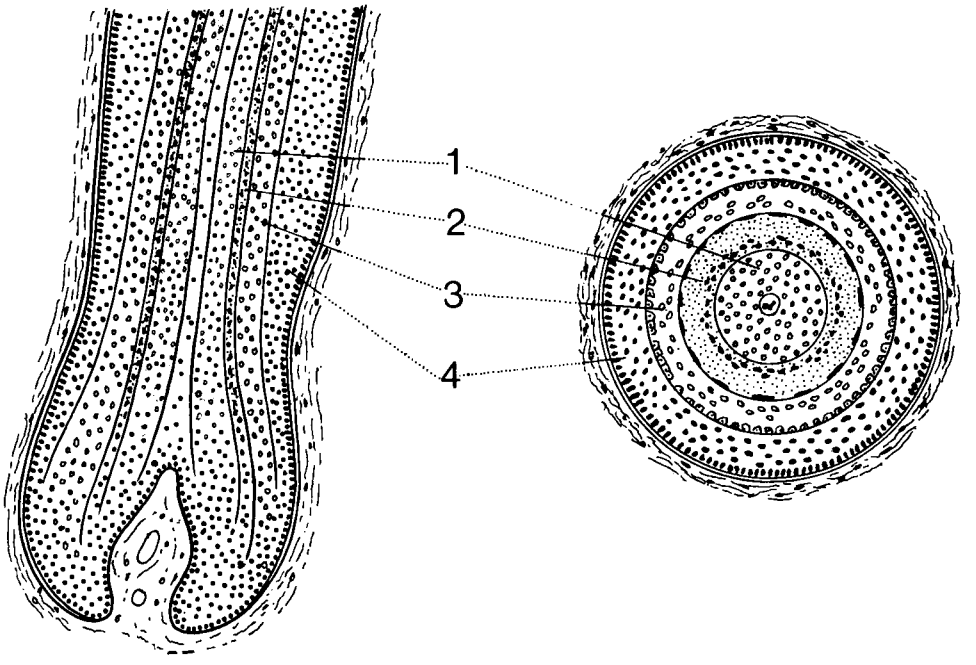


FIG. 17.13.—Diagram of a hair follicle in longitudinal section (left) and in transverse section (right). 1, cortex of hair; 2, cuticle of hair; 3, layers of Huxley and Henle; 4, outer root sheath.

The colour of the hair depends on the amount of pigment and of air present. Much air makes the hair look dark by transmitted light and white by reflected light: much pigment produces a dark effect with both kinds of light. Loss of pigment makes the hair look grey: if these hairs happen to possess much medullary substance the loss of pigment produces a bright silvery effect.

The *root* of the hair is enlarged into a hair-bulb which consists of growing cells and fits over the vascular papilla. This papilla consists of connective tissue, blood vessels and nerves, and projects upwards into the bottom of the follicle; it corresponds to an ordinary dermal papilla.

Structure of the Hair Follicle (Fig. 17.13)

The follicles of all but the finest (lanugo) hairs contain both epidermal and dermal components: the epidermis provides the root sheaths and the dermis the connective tissue sheath.

(1) **Root Sheaths.** The *inner root sheath*, closely investing the hair below the level of the entrance of the sebaceous gland into the follicle, is derived from the stratum corneum and the other epidermal layers: three layers can be distinguished, the cuticle (which lies next to the cuticle of the hair), Huxley's layer composed of 1–3 layers of keratinized cells, and an outer single-celled Henle's layer. The *outer root sheath* is derived from the stratum germinativum and is continuous with it. Towards the papilla at the bottom of the follicle the sheaths blend into a mass of cells (the *matrix*) from which the hair and its sheaths grow.

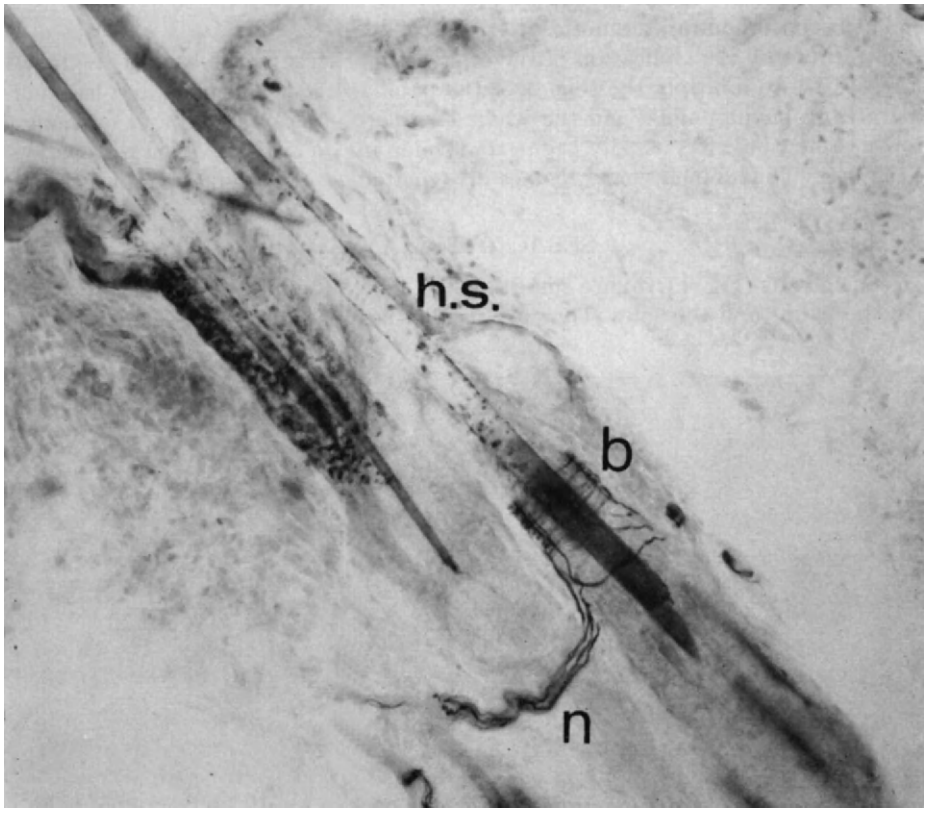


FIG. 17.14.—A section of skin which passes through a hair follicle. The preparation has been made by a silver technique which reveals the nerve fibres. The hair shaft (h.s.), the nerve fibre (n) and the basket-like arrangement of nerve fibres (b) around the shaft may be seen.

(2) **Connective Tissue Sheath.** The dermis adjacent to the invaginated epithelial portion is condensed into another sheath. It consists of a vascular layer (corresponding to the vascular papillary region of the dermis) separated by a hyaline membrane (the *glassy membrane*) from the external root sheath: each hair sac has its own blood vessels. In some of the large tactile hairs and whiskers the veins of this layer are enlarged at the bottom of the follicle into a kind of erectile tissue. In these hairs also the nerves are particularly abundant forming a basket-like arrangement around its base (Fig. 17.14).

Muscle of the Hair

A band of smooth muscle is attached to the middle of the connective tissue sheath of the follicle, the other end of the muscle terminating in the region of the dermal papillae. The

contraction of this muscle causes at first a more upright position of the hair, then invagination of the surface where it is attached and a raising of the surrounding region by pulling up the follicle, producing "goose flesh". Contraction of the muscle usually causes extrusion of the secretion of the adjacent sebaceous gland.

Growth of the Hair

Hairs grow for a definite period and are then replaced: in some animals that shed their coats the replacement is very rapid. In man the scalp hairs are replaced every two to four years, growing about five inches a year, and the eyelashes every three to five months, but the replacement is continuous and unnoticed.

The hair grows by multiplication of the cells of the matrix at the base of the root sheath. When these cease to multiply the cells over the papilla become cornified and the root of the hair separates from the papilla and the whole structure moves upward and falls out. Before this happens a new hair has usually begun to develop in the same sac from a growing mass of cells at the base of the follicle that becomes invaginated by the original papilla.

SEBACEOUS GLANDS

Sebaceous glands (Fig. 17.15) are small sac-like glands found in the skin all over the body except in the palms and the soles. They are usually associated with the hairs, one or several

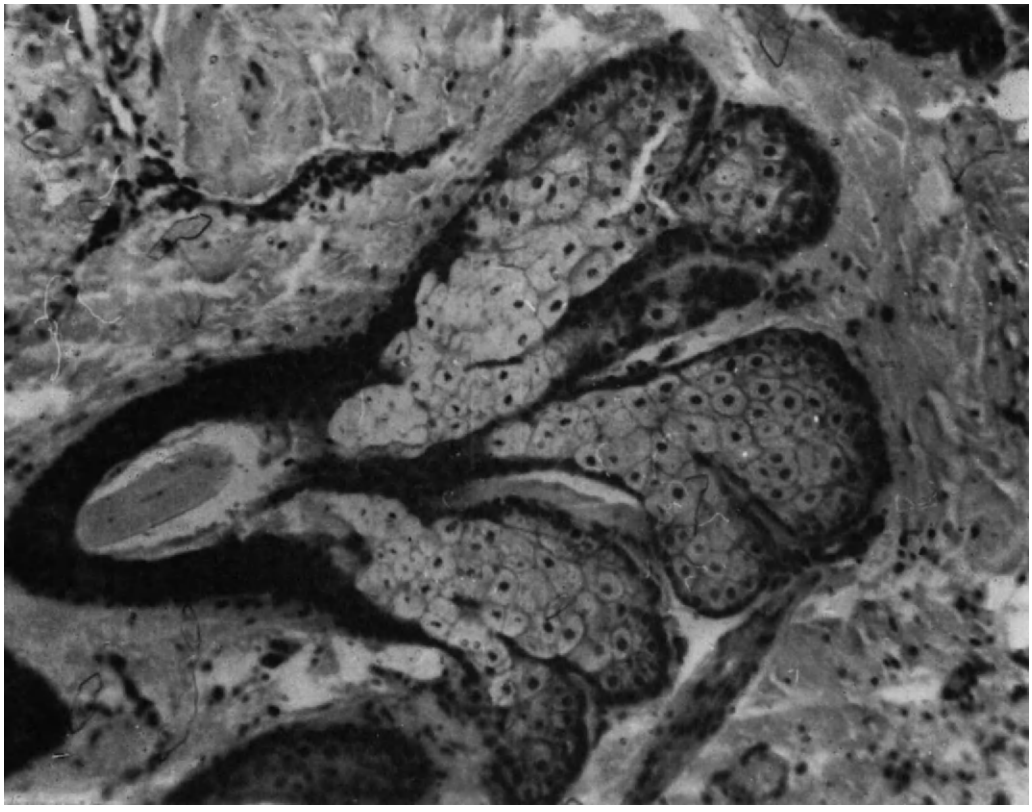


FIG. 17.15.—Sebaceous glands in the dermis of the skin; these would open into a hair follicle at the left of the micrograph. The light appearance of the cells is due to loss of their lipid during the preparation of the section.

80 μm.

glands opening into one hair follicle. They occur independently of hairs in the lips, at the corners of the mouth, in parts of the external genitalia, and as tarsal (Meibomian) glands in the eyelids.

The glands are alveolar, being simple or branched: the alveoli open into a very short, wide duct lined by stratified epithelium. The alveolus is enclosed by a basal lamina: the cells are usually polyhedral and fill the whole alveolus. Secretion occurs by a disintegration of the entire cell, and is therefore called *holocrine*. The centrally placed cells show the greatest change: replacement of the cells occurs by division of the cells near the ducts and the moving inwards of the new cells. Outside the basal lamina is a rich network of blood capillaries: lymphatic capillaries are absent.

DEVELOPMENT OF THE SKIN AND ITS APPENDAGES

The epidermis is of ectodermal origin: it is at first only one cell deep, but within three or four weeks it differentiates into two layers. The outer layer (epitrichium) thickens, and many of the cells subsequently become vesicular and are later shed, mixing with the secretion of the

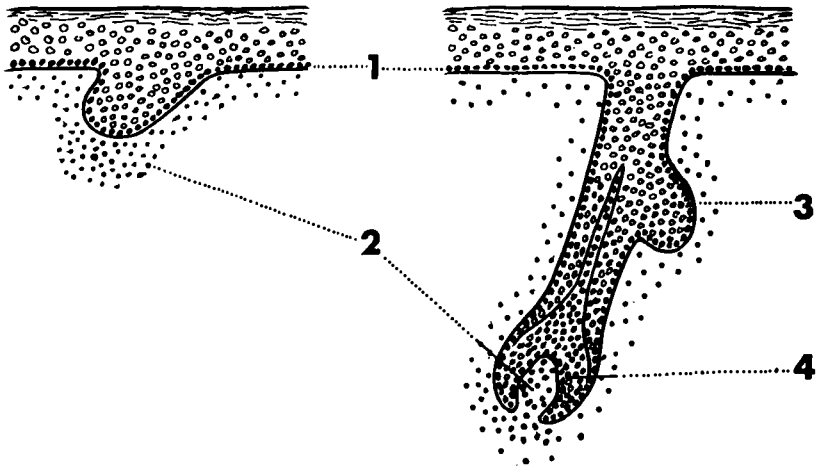


FIG. 17.16.—A diagram to show the early development of a hair follicle. 1, stratum germinativum; 2, dermal hair papillae; 3, rudiment of sebaceous gland; 4, hair root.

sebaceous glands to form the *vernix caseosa* of the foetus: this waxy covering serves, as a protection against the amniotic fluid. The cells intervening between the outer and inner layers become flattened and then cornified and give rise to the stratum corneum. The inner layers become many cells thick, giving rise to the stratum granulosum and stratum spinosum, with the stratum germinativum as the deepest row of cells. Pigment granules appear after birth.

The boundary between epidermis and underlying connective tissue is at first smooth: about the third foetal month solid downgrowths of the epidermis begin to appear (Figs. 17.16, 17.17): these give rise to the hairs, appearing first in the region of the eyebrows and chin. The base of the hair comes to invest the papilla of underlying dermis and the whole pushes deeply downwards. The first formed fine hairs constitute the *lanugo*: these are shed before or soon after birth and new hairs take their place. The sebaceous glands arise from the hair follicles during the fourth foetal month.

The sweat glands are developed in the same way as the hairs (Fig. 17.18), but are ultimately coiled at the extremity and differentiated into hollow tubes. They first appear early in the fifth foetal month and are initially confined to the palms and the soles.

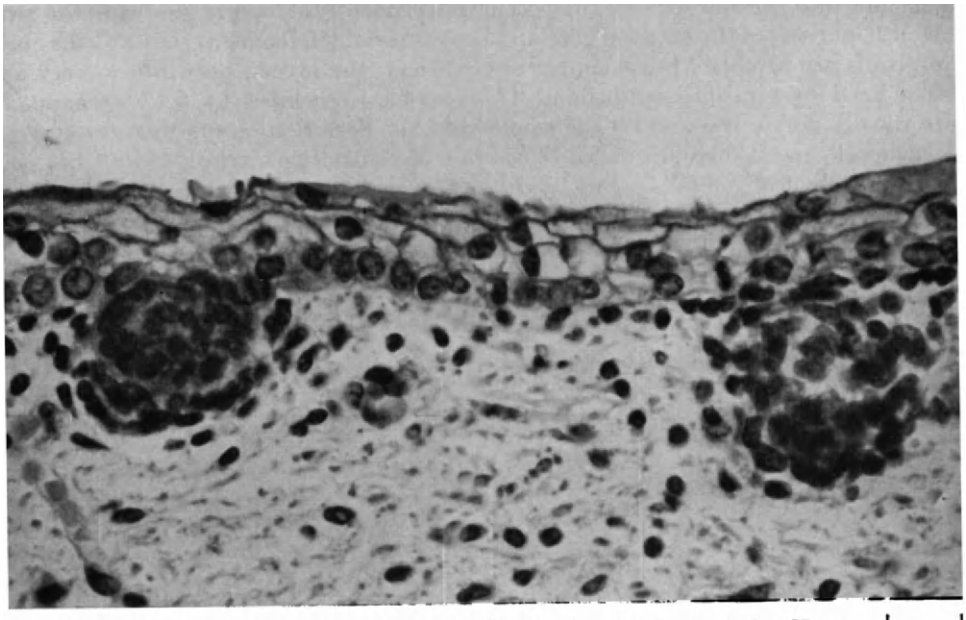


FIG. 17.17.—Micrograph of a section of foetal skin showing developing hairs. Note that the typical stratification of the epidermis is just beginning. 20 μm.

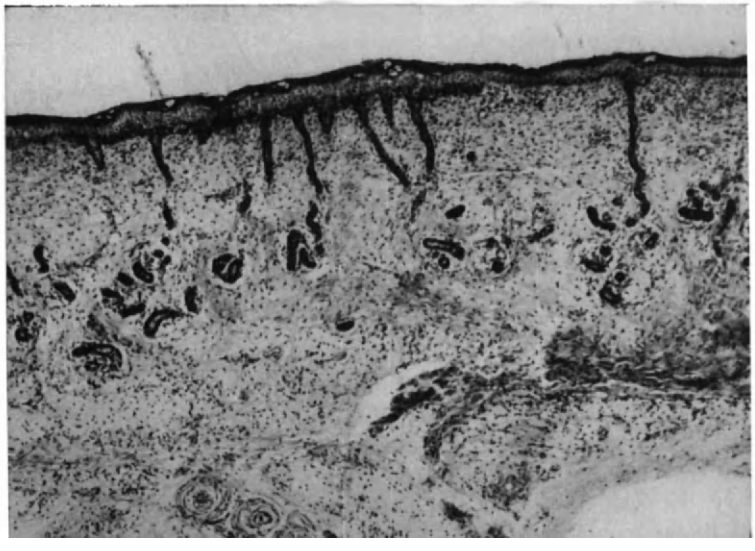


FIG. 17.18.—Section of the skin of a foetus *c.* 22 weeks. This shows the developing sweat glands in the dermis. 500 μm.

The nails first appear as a thickening of the stratum lucidum about the third foetal month. They become free about the sixth month, gradually growing forward and becoming thicker: they grow more quickly on the fingers than on the toes.

SPECIAL MODIFICATIONS AND REACTIONS OF THE SKIN

Blisters

Fluid often collects in relatively large quantity between the cells of the epidermis, as a result of some inflammatory condition or of actual trauma (usually by friction). If the fluid contains blood then the dermis must be involved as well as the epidermis, as the latter contains no blood vessels.

Pigmented Naevi

These are soft congenital warts in the skin. They consist of groups of cells, many containing pigment, lying as "nests" beneath the epithelium: their origin, whether epidermal or mesodermal, is disputed.

Age Changes

Several well-defined changes occur in the skin in older persons. The dermal papillae largely disappear, the dermis is reduced in vascularity and sweat and sebaceous glands atrophy. Hair follicles become smaller and fewer in number. The elastic fibres of the dermis have largely disappeared and the connective tissue matrix has lost some of its high degree of hydration so that the skin tends to be wrinkled and flabby.

In the epidermis there is a slowing of the mitotic rate in the stratum germinativum and hence the layers of the epidermis tend to be thinner and less well developed than in the young person. As a result of this latter change, fissures appear in the epidermis so that micro-organisms may more easily penetrate the skin. There is a general trend towards a lesser resistance to mechanical injury and small wounds are more easily formed and heal less easily.

Repair of Skin and Skin Grafting

When an area of epidermis has been removed (as by a blister) repair is effected by growth from the epidermis. If a large area has been denuded the repair is slow, as the new skin has to grow in from the epidermis at the margins of the wound. In skin grafting small pieces of healthy skin (including the deep layers of the epidermis) are transplanted on to the bare area, and each graft that "takes" serves as a centre from which the new epidermis spreads outwards, so that the bare surface is quickly covered.

References for further detailed study:

A. S. Breathnach, "Melanin Pigmentation of the Skin", Oxford Biology Readers, No. 7 (1971).
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CHAPTER 18

RESPIRATORY SYSTEM

The respiratory system consists of a series of passages connecting the essential respiratory organs (the lungs) with the exterior.

(*a*) **The Conducting Portion** includes:

(1) The hollow passages of the nose, communicating with the outside air, and leading by the nasopharynx into

(2) The *pharynx*. This connects the mouth with the oesophagus, and also connects the nasal cavity with

(3) The *larynx*, which contains the vocal cords. The larynx leads into

(4) The *trachea*, which divides and, in its turn, leads to

(5) The *bronchi*: these diminish in calibre with each bifurcation, the smallest tubes being known as

(6) The *bronchioles*.

(*b*) **The Respiratory Portion** includes:

(1) *Respiratory bronchioles*

(2) *Alveolar ducts*

(3) *Alveolar sacs*

(4) *Terminal alveoli*

As the lungs are thus in free communication with the outside air they are especially liable to damage from inhaled dust and to bacterial infection and to the spread of such infection, particularly as there is continuity of mucous membrane throughout. Several defensive mechanisms normally keep the alveoli free from damage and infection, namely, the presence of diffuse lymphoid masses immediately beneath the epithelium throughout the tract, the presence of mucin (from mucous glands) combined with the activity of the ciliated epithelium driving all foreign particles outward and the presence in the alveoli of phagocytic cells.

THE NOSE

The nasal passages are divided into the vestibule opening to the exterior, the nasal cavity proper and the small olfactory region.

(*a*) Vestibule

The epithelium is stratified and continuous with that of the adjacent epidermis. The underlying stroma is very cellular and there are large sebaceous glands: the hairs (setae) are long.

(*b*) Nasal Cavity

The mucous membrane consists of a pseudostratified, columnar, ciliated epithelium with interspersed goblet cells many of which lie in small crypts, resting on a basal lamina. The underlying connective tissue is thick and directly connected with the periosteum and perichondrium of the bony and cartilaginous walls of the nasal cavity. The submucous layer contains smooth muscle, lymphoid nodules and simple serous and mucous glands. Particularly in the region of the conchae this layer also contains networks of very large thinwalled veins which serve to warm and humidify the inspired air (see p. 190).

The accessory nasal sinuses have a similar but much thinner mucous membrane.

(*c*) **Olfactory Region**

The epithelium is thick and of the pseudo-stratified, columnar type (Fig. 18.1): there are three kinds of cells:

(1) **Olfactory Cells** (Fig. 18.2). These are bipolar, fusiform nerve cells, with round nuclei deeply placed: the dendritic process extends to the surface and ends in a few minute hairs. These hairs have the characteristic ultrastructure of cilia and are probably slightly motile. (In the frog, these cilia are 150–200 μm. long and usually bend so that they are parallel to the surface of the epithelium.) The basal end continues as an unmyelinated nerve process which passes through the cribriform plate and enters the olfactory bulb.

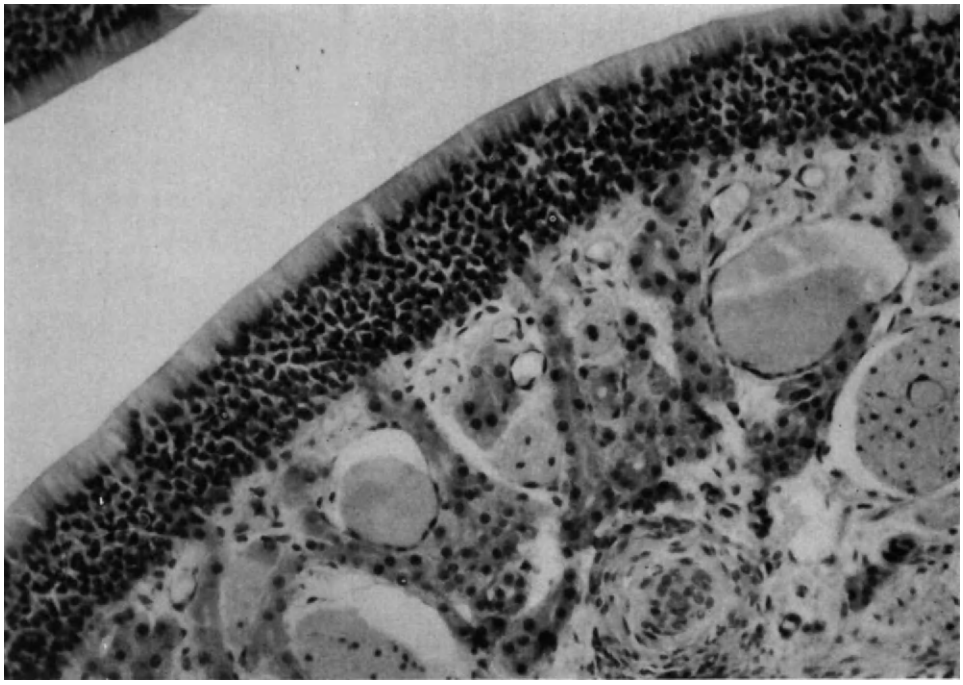

FIG. 18.1.—The olfactory mucosa; the pseudostratified nature is evident but the cell types present cannot be differentiated without the use of special staining techniques. Note the extensive vascularity of the submucous layer.

65 μm.

(2) **Supporting (Sustentacular) Cells.** These are cylindrical, granular cells with oval nuclei and a microvillous free border: the deep end of the cell is often forked. (In many animals, but not in man, these cells are ciliated.) The E/M has shown that these cells are attached by a well-developed junctional complex (p. 40) to the adjacent sensory cells.

(3) **Basal Cells.** These lie between the bases of the other cells and are usually conical and darkly staining.

Beneath the epithelium the connective tissue of the lamina propria contains the olfactory tubulo-alveolar serous glands (of Bowman) providing a continuous cleansing secretion, the bundles of olfactory nerve fibres, and a very rich plexus of blood capillaries. Deeper still is found a plexus of large veins and also a network of lymphatic capillaries continuous with the large lymphatics of the sides of the head and apparently with the sub-arachnoid space: this latter provides a potential pathway for spread of infection from the nose to the meninges.

There are, in addition, myelinated nerve fibres of the trigeminal nerve, that end in free arborisations among the sustentacular cells, and are receptors for stimuli other than odours.

The *nasopharynx*, conveying the air from the nasal cavity to the pharynx, is lined by columnar, pseudo-stratified, ciliated epithelium. Beneath the epithelium is a layer of connective tissue with many longitudinal elastic fibres, and outside this is a layer of muscle. There are mixed serous and mucous glands, and much lymphoid tissue, both diffuse and aggregated into the naso-pharyngeal tonsil (p. 285).

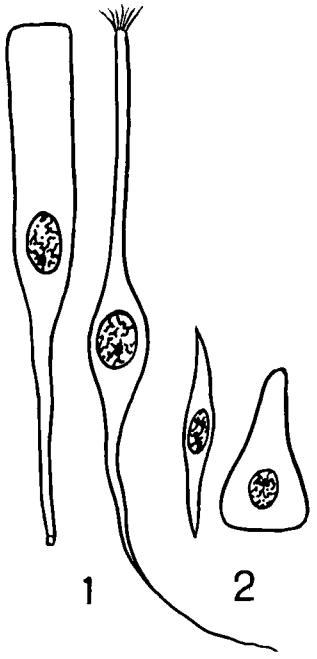

FIG. 18.2.—A diagram of the cell types in the olfactory mucosa. 1, supporting cell (on the left) and olfactory cell; 2, basal cells.

THE LARYNX (Fig. 18.3)

The larynx consists of a framework of cartilages joined by fibrous bands, and the resulting tube is lined by a mucous membrane. The extrinsic muscles are concerned with swallowing, and the intrinsic muscles alter the size and shape of the tube and are therefore concerned with speech. Of the cartilages, the thyroid, cricoid and main part of the arytenoids are of the hyaline variety, while the epiglottis, part of the arytenoids and the corniculate and cuneiform cartilages are elastic: the hyaline cartilages are subject to calcification in old age.

The epithelium lining the larynx is for the most part pseudo-stratified, columnar and ciliated, the cilia beating towards the mouth: there is a definite basal lamina: the vocal folds and most of the epiglottis, however, are covered by a stratified squamous epithelium. Goblet cells are numerous. Diffuse lymphoid tissue and mixed glands are found in the deep connective tissue, which latter merges into the perichondrium of the cartilages.

The *epiglottis* (Fig. 18.4) contains a central core of elastic cartilage and is covered by stratified epithelium: some taste buds are usually present.

The *vocal folds* consist of the upper free edge of a fibro-elastic lamina (crico-vocal membrane) covered by a stratified squamous epithelium, which gives place to a ciliated epithelium over the infra-vocal part of the membrane. The adjacent muscle is concerned in the alterations in shape of the space between the folds and in their relaxation and tightening.

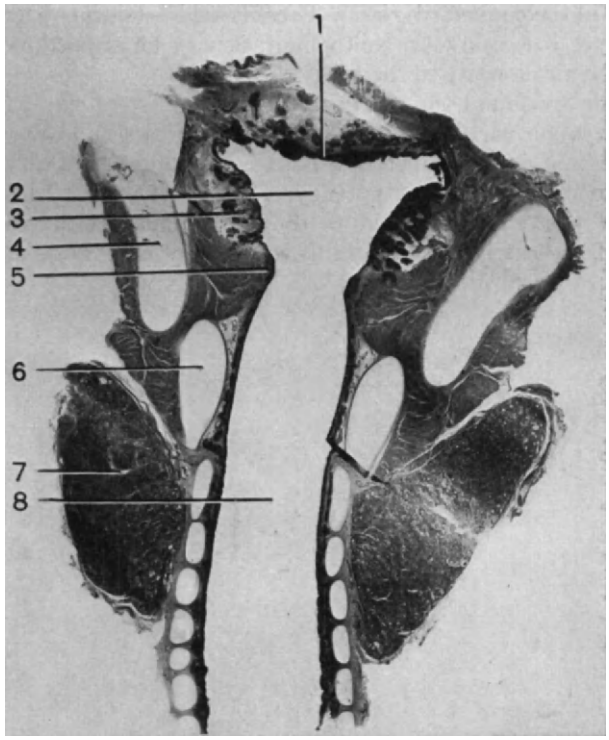

FIG. 18.3.—Coronal section through the larynx of a child; 1, epiglottis; 2, vestibule of larynx; 3, mucous and serous glands; 4, thyroid cartilage; 5, vocal fold; 6, cricoid cartilage; 7, lobe of the thyroid gland; 8, trachea.

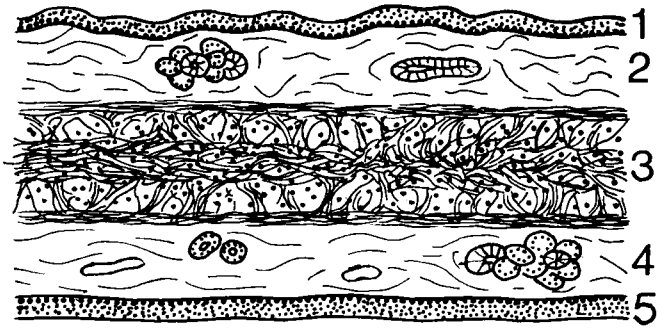

FIG. 18.4.—Diagram of the structure of the epiglottis. 1, stratified epithelium on the lingual surface; 2, glands in the connective tissue; 3, elastic cartilage; 4, glands in the connective tissue on the tracheal surface; 5, stratified epithelium of tracheal surface.

TRACHEA, BRONCHI AND BRONCHIOLES (FIGS. 18.5, 18.6)

The *trachea* is a thin-walled, rigid tube, lined by a ciliated, pseudo-stratified, columnar epithelium, with basal supporting cells and many goblet cells, and resting on a definite basal lamina containing reticulin fibres. Beneath the epithelium is a layer of connective tissue containing large numbers of elastic fibres, simple branched mucous and serous glands (to provide mucin and moisture) opening into the lumen by short ducts, and diffuse masses of lymphoid tissue: the glands are particularly numerous at the back. Eosinophil cells are of

frequent occurrence in the connective tissue. There is a well-developed network of veins in the connective tissue layer just below the epithelium, serving an important role in the warming and moistening of the air en route to the lungs.

Outside this layer are found the cartilages. These consist of sixteen to twenty C- or Y-shaped masses of hyaline cartilage obliquely encircling the tube except posteriorly, and embedded in moderately dense connective tissue containing many elastic fibres. They play an important rôle in maintaining the potency of the trachea at all times. In old age they become fibrous. The posterior wall, next to the oesophagus, has no cartilage, but the gap between the ends of the cartilaginous rings is bridged by a network of smooth muscle and

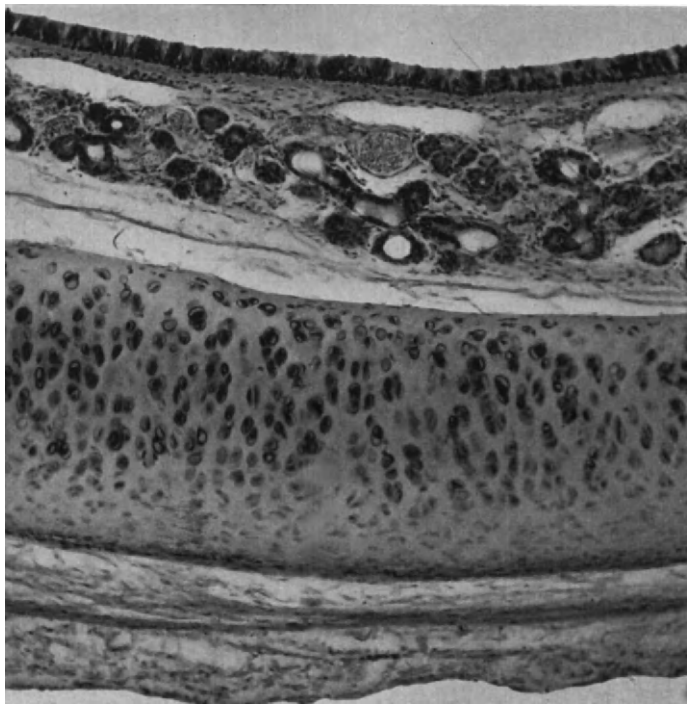

FIG. 18.5.—Section of the wall of the trachea; the lumen is at the top of the micrograph. Note the numerous glands in the submucous connective tissue and the hyaline cartilage ring.

30 μm.

fibrous tissue running for the most part transversely (Fig. 18.7). Outside this layer again is some loose connective tissue continuous with the surrounding structures.

The trachea divides into two main *bronchi*, one on each side, which enter the lung at the hilum: they then bifurcate repeatedly, giving rise to *bronchial tubes* (*bronchioles*) of decreasing calibre.

The bronchi are arranged in a definite pattern, each group supplying one of the so-called *broncho-pulmonary segments*. A knowledge of these is of great importance in clinical work; details of their arrangement may be found in any of the larger text books of anatomy.

The two *main bronchi* have the same structure as the trachea.

The intrapulmonary bronchioles decrease in size on repeated bifurcation, and the following changes occur in their structure as they become smaller (Fig. 18.8).

(1) The *epithelium* becomes thinner and is reduced to a single layer of columnar ciliated cells: as the tube becomes smaller still the cells become lower, and in the smallest respiratory

bronchioles they are cubical and not ciliated. Goblet cells secreting mucin are found in all but the smallest tubes; their number is increased by moderate irritation. The bronchioles are kept from being blocked by excessive mucin by means of the cough reflex and by the activity of the cilia on the epithelial cells.

(2) The *lymphoid tissue* increases as the tube becomes smaller, and infiltration of the epithelium is common: the lymph nodes near the root of the lung are often pigmented, due to accumulation of carbon particles removed from the air by the action of "dust cells" (p. 269). Collections of lymphoid tissue are particularly well developed at the bifurcations of the tubes, and where the alveolar passage ends in the atrium.

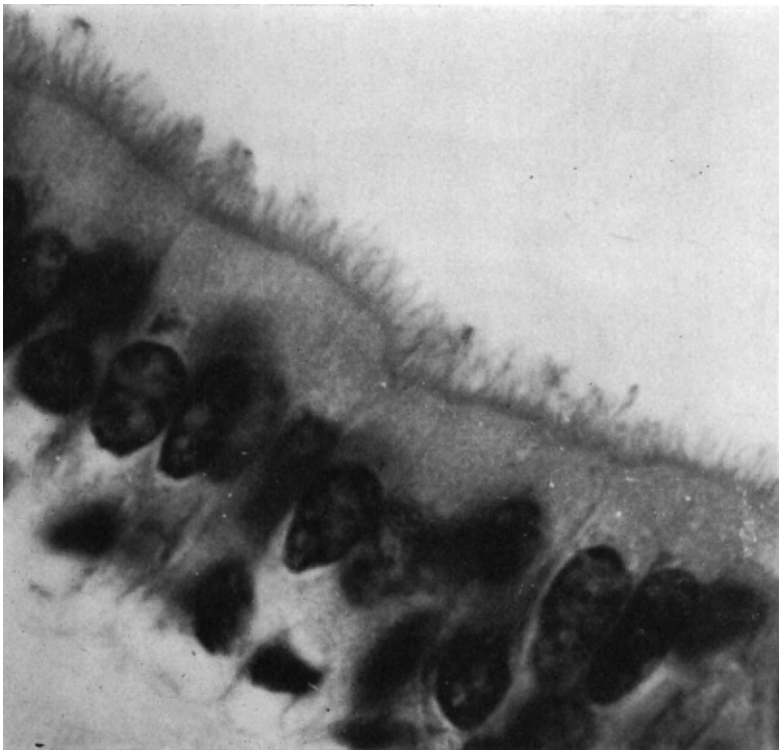

FIG. 18.6. The tracheal epithelium, showing the ciliated cells. 5 μm.

(3) There is considerable development of *smooth muscle* between the epithelium and the cartilage: the relative thickness of the muscle coat is greatest in the medium-sized bronchi, and it then diminishes in amount with diminishing calibre of the tube. Most of the muscle is circular in arrangement, but obliquely running bundles of fibres help to make a kind of reticulum. Many elastic fibres are found in the muscle network, and also in the outermost layer of connective tissue. There is a sphincter at the opening of the alveolar passage into the atrium, and no muscle beyond this point.

(4) The *cartilage* appears as disconnected plates in sections; in reality the cartilages are continuous rings of a very irregular shape. The cartilage becomes progressively less in amount until in the small bronchial tubes it is completely absent.

(5) The *glands* gradually diminish and are absent from the bronchioles that are devoid of cartilage.

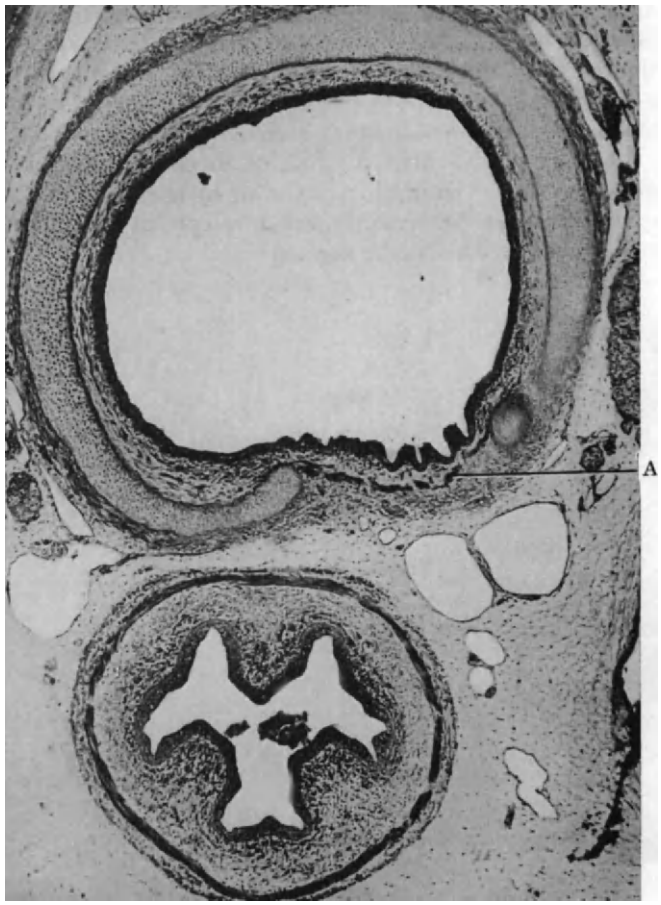

FIG. 18.7.—Transverse section of the trachea and oesophagus of an 18 week human foetus. The trachea, at the top of the picture, shows the posterior gap in the cartilage ring bridged by the smooth muscle fibres of the trachealis muscle (A).

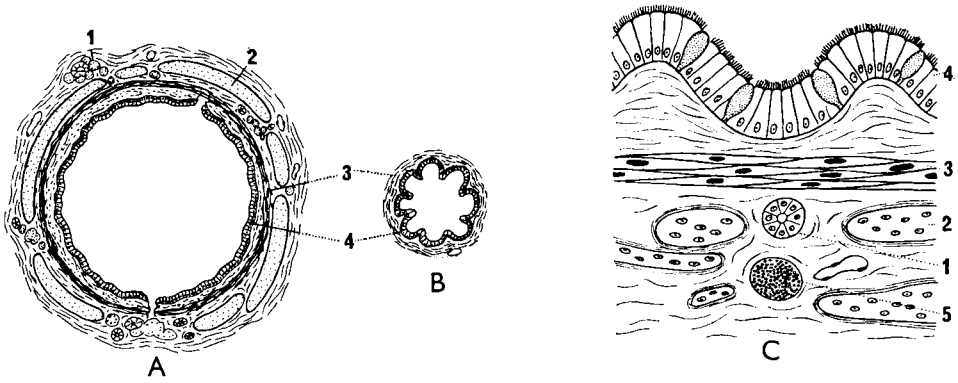

FIG. 18.8.—A diagram of the structure of the bronchiole walls. A, medium size; B, small size. Note the absence of cartilage in the small bronchiole. C represents a magnified view of part of the wall of a large bronchiole. 1, serous and mucous glands; 2, cartilage; 3, smooth muscle; 4, pseudostratified epithelium with goblet cells; 5, nerve in T.S.

Blood Vessels and Lymphatics

The large vessels are found in the submucous layer, with capillary networks in each coat. The lymphatics pass to the hilar lymph nodes, and to those that accompany the main bronchi and trachea.

Nerves

A fine sub-epithelial plexus receives fibrils from among the epithelial cells and gives rise to the myelinated sensory fibres. Autonomic parasympathetic ganglion cells are scattered in the walls, and from these post-ganglionic fibres pass to the glands and to the smooth muscle.

THE LUNGS

The general plan of the lungs is somewhat like that of a compound alveolar gland, the conducting portion (of trachea and bronchi) corresponding to the ducts, and the respiratory

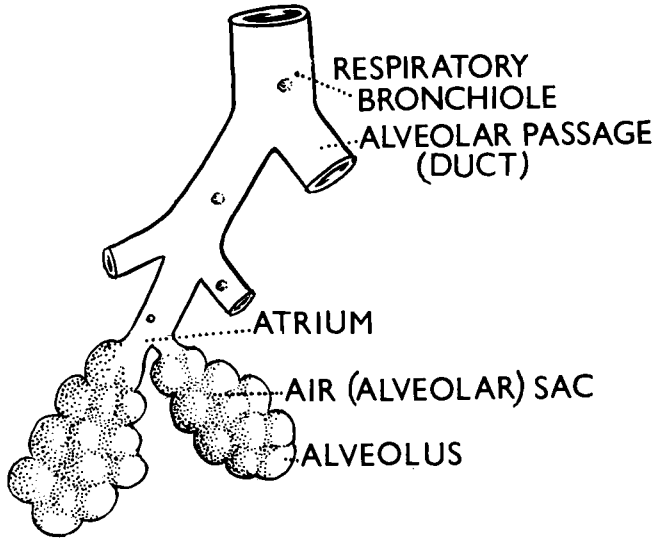

FIG. 18.9.—A diagram to illustrate the terminal divisions of the bronchioles and the respiratory portions of the lung.

portion (of air sacs and alveoli) corresponding to the secreting alveoli (Fig. 18.9). This arrangement can be seen in a section of an early embryonic lung, but in the fully-developed condition the resemblance is not so clear.

The surface of the lungs is covered by a serous membrane, the *visceral pleura*, which at the root of the lungs is reflected upon the inner surface of the chest wall as the *parietal pleura*. Septa of connective tissue pass from the visceral pleura into the organ dividing it into lobules.

A *functional pulmonary unit* can be regarded as consisting of the respiratory bronchiole with its alveolar ducts leading into the atria with their air sacs studded with terminal alveoli, together with the blood vessels, lymphatics, nerves and connective tissue that supply them (see diagram Fig. 18.9).

A microscopical section of the lung (Fig. 18.10) shows a network of empty spaces surrounded by thin walls, and containing portions of walls of various sized bronchi, bronchioles and blood vessels. If the section is thick it is easy to see that the structure is really like a honeycomb with the air sacs and alveoli as the "cells", and containing bronchioles into which the atria and air sacs open. (Histological preparations are apt to give a distorted picture owing to the enormous

elastic recoil that occurs when the lungs are removed from their usual environment of negative pressure. It must also be remembered that during normal respiration the lungs undergo very considerable alteration in volume and in internal configuration.)

The *respiratory bronchioles* are continuations of the small bronchi: the epithelium changes from columnar ciliated to cubical and non-ciliated. The wall consists of fibrous tissue containing some circularly- and spirally-arranged smooth muscle and elastic fibres; no cartilage, mucous glands or lymph nodes are present.

The *alveolar ducts* (*atria*) are thin-walled and like the respiratory bronchioles except that their epithelium is flat and of the respiratory type (see below). Numerous *alveolar sacs* and

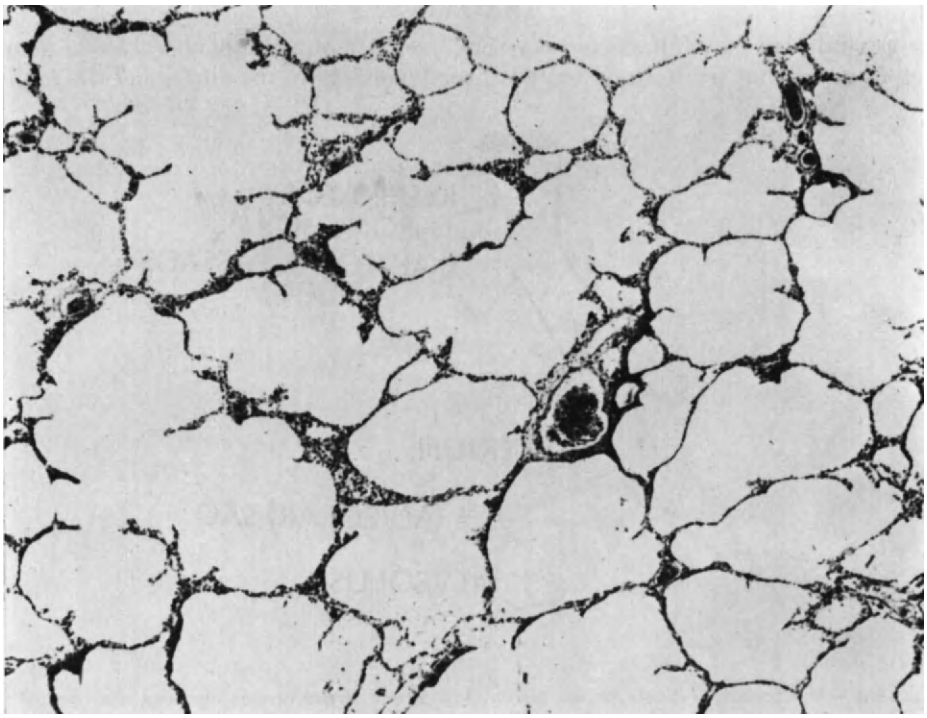

FIG. 18.10.—A micrograph of a section of the lung. The air sacs and the alveoli give the characteristic "empty" appearance to such sections.

single alveoli open off the alveolar ducts. There is no muscle in the walls of the alveolar sac, but the muscle of the alveolar duct makes a kind of sphincter at the junction between them.

The *alveoli* are very thin-walled blind sacs that open on one aspect into the alveolar sacs. (Alveoli temporarily not in use have a small lumen and a thick wall.) The walls between the alveoli (Fig. 18.11) are extremely thin and consist of a stroma containing enormous numbers of anastomosing capillaries, supported in a network of fine reticular and elastic fibres (Fig. 18.12), the latter being continuous with the elastic membranes of the bronchioles. In addition, both the reticular fibres and the elastic fibres are in continuity with those in the walls of the blood vessels. In the walls also are found some macrophages and some fibrocytes. *Alveolar pores*, 8–10 μm. in diameter, are present in the inter-alveolar septa between adjacent alveoli: such holes would help to prevent collapse or overdistension of the alveoli if a small bronchiole became occluded. The nature of the cells lining the alveolar walls has been much discussed in the past. It was suggested that the lining was incomplete and consisted of small groups of

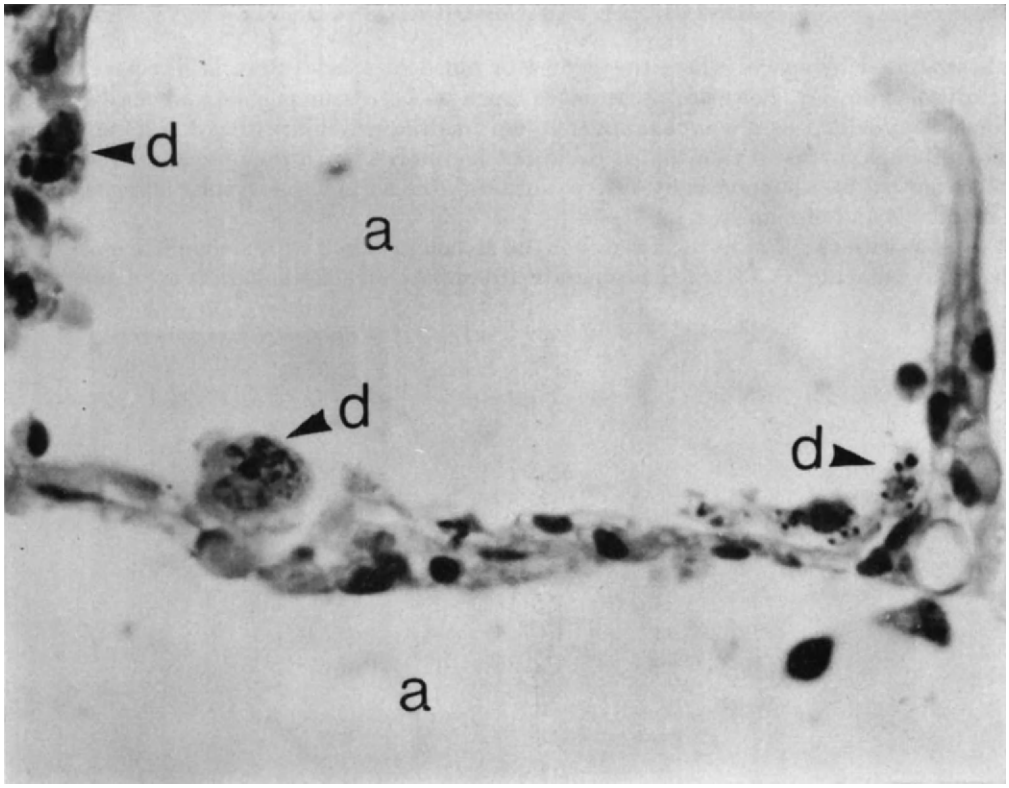

FIG. 18.11—A high power micrograph of part of an alveolar wall which supports the air-spaces (a) of two adjacent alveoli. Dust spaces (d) with their prominent inclusions of phagocytosed particles are seen in the alveolar wall.

10 μm.

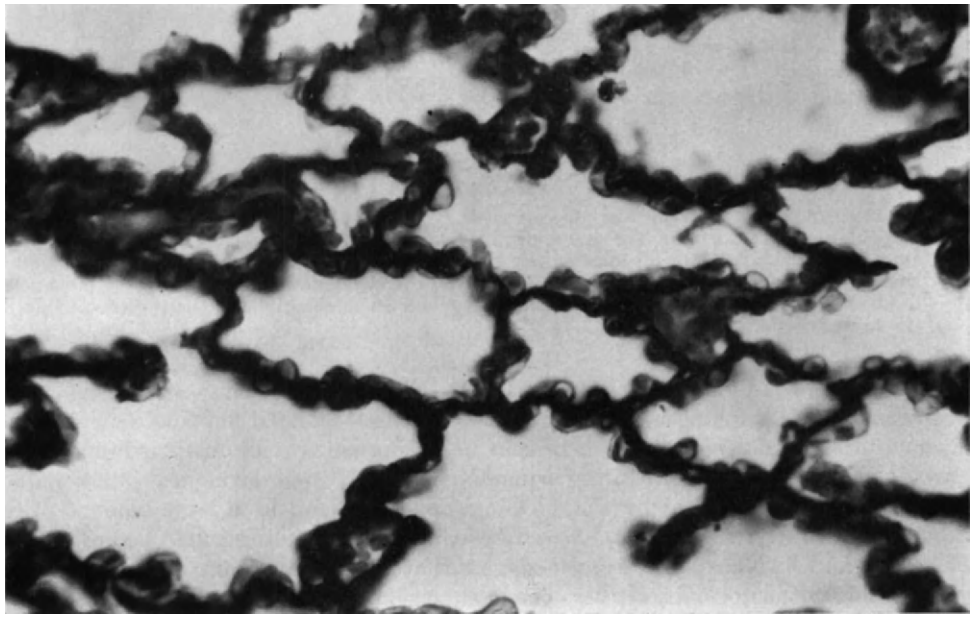

FIG. 18.12.—A preparation of the alveolar walls to show their content of elastic fibres (here stained darkly).

15 μm.

cells separated by spaces where the stroma or blood capillaries were in direct contact with the alveolar air. As, however, an exudate (such as occurs in pneumonia) may lift off the alveolar epithelium as a complete layer, there was the possibility that the lining was complete although it was so thin that it could not be resolved with the optical microscope. This latter view has been supported by observations with the E/M on the alveolar lining of a number of mammals and of man.

Studies with the E/M have shown that the alveoli are lined with a complete epithelium of squamous cells (Fig. 18.13) which is separated from the thin endothelium of the capillaries by a

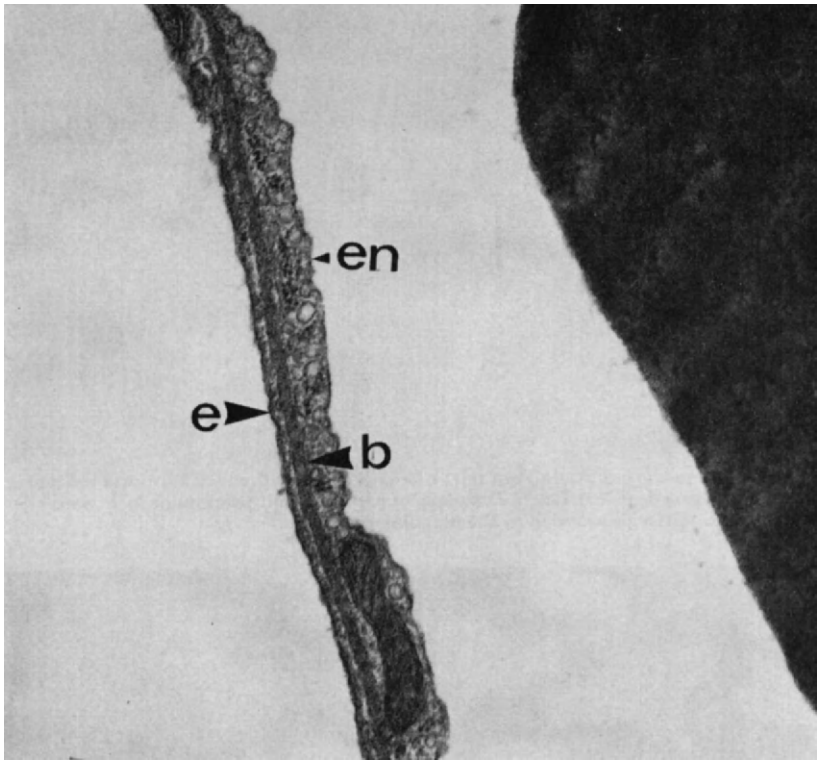

FIG. 18.13.—An E/M of part of the alveolar wall. The lumen of the alveolus is at the left of the micrograph. Note the extreme thinness of the squamous alveolar epithelium (e) and the close relationship with the equally thin endothelial cell of the capillary wall (en) and the basal lamina (b). The large structure at the right of the micrograph is part of a red blood corpuscle. None of the surfactant lining the alveolus has been preserved.

1 μm.

homogeneous basal lamina. The epithelial lining cells of the alveolus are sometimes called *small alveolar cells*. In amongst these latter cells one finds isolated cuboidal or *great alveolar cells* (sometimes called *septal cells*); these cells have short microvilli on their luminal surface and are joined to their neighbouring squamous cells by well-developed junctional complexes. They are characterized by a large Golgi apparatus and by a large amount of rough surfaced endoplasmic reticulum and free ribosomes. Many multilamellar bodies are found in these cells and histochemical studies suggest that these are rich in phospholipids which are very soluble in the normal fixatives. The loss of the multilamellar bodies on fixation might well account for the presence of the numerous vacuoles which are seen in these cells in normal histological preparations. It seems probable that the multilamellar bodies are the source of

the surface active layer (*surfactant*) which is known to line the alveoli and which because of its low surface tension helps to stabilize the alveolus and prevent it from collapsing.

The large phagocytes that are usually seen free in the alveoli or attached to the walls are in all probability derived from cells situated between the capillary walls and the alveolar lining. It is known that cells having phagocytic properties exist in this position. These "dust-cells" (Fig. 18.11) as they are called are particularly numerous in the lungs of town dwellers and are important in removing from the alveoli the smallest particles (< 1 μm) of dust which have not been trapped by the bronchiolar mucous.

Blood Vessels

The lungs receive a double blood supply, the bronchial system carrying blood for the nutrition of the bronchial tubes and lung tissue, and the pulmonary system carrying blood for the respiratory exchanges in the lungs. The bronchial vessels arise from the aorta or

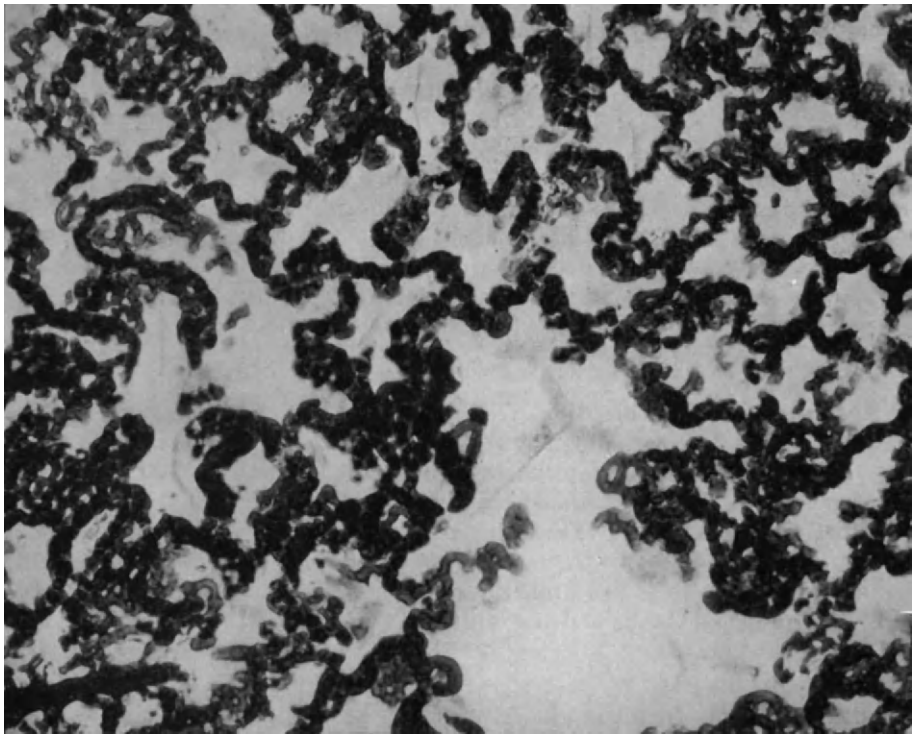

FIG. 18.14.—A section of lung with the capillaries injected with carmine/gelatin. Several alveoli are cut tangentially and show the rich vascularity of their walls. 30 μm.

intercostal arteries, and follow the bronchi, running within their fibrous coats to be distributed to their glands and walls and to the connective tissue of the pleura. The pulmonary vessels are derived from the pulmonary arteries, and also follow the bronchi, running in the connective tissue outside their walls. On reaching the respiratory bronchioles the arteries furnish a branch to each alveolar duct; this breaks up into a capillary network over all the communicating alveoli (Fig. 18.14). There is capillary anastomosis between the bronchial and pulmonary systems, the venules ultimately draining away to the pulmonary veins. The pulmonary vessels are peculiar in that their adventitial coat consists of a system of communicating cavities filled with lymph instead of the usual fibrous coat (see below).

Lymphatic Vessels

The lungs have two groups of lymphatics, one group belonging to the pleura and the other to the lung tissue; both drain into the large lymph nodes at the hilum of the lung. The *pleural lymphatics* form a dense network and the vessels are provided with many valves to direct the flow towards the root of the lung. The *pulmonary lymphatics* include those supplying the bronchial tubes as far as the alveolar ducts where they end in plexuses round the blood vessels, and those accompanying the branches of the pulmonary artery and vein: there are no lymphatics in the walls of the atria or alveoli. In addition to these lymphatics, there is a vast system of communicating intra-adventitial spaces in the walls of the bronchioles, arteries, veins and lymphatics: these spaces are separated by interlacing collagen and elastic bundles and pass into the true lymphatic capillaries. The movements of respiration cause the lungs to act like a lymph heart: at inspiration the intra-adventitial spaces and all the lymphatic system are filled with lymph from the blood capillary system and lymph nodules, while at expiration the lymph is carried away from the lung. The pulmonary lymphatics have no valves except in a few vessels that connect the pleural and pulmonary systems: these valves direct the flow toward the pleura so that lymph can pass from lung tissue into the pleural lymphatics if there is interference with its normal flow toward the hilum.

Lymphoid Tissue

This may be found in the connective tissue round the bronchi, arteries, veins, and in the pleura. Definite lymph nodes are associated with the larger divisions of the bronchi and the vessels: there are none in the pleura. The smaller lymphoid aggregations can also act as filters in the lymph circulation, and as the centres to which phagocytes carry any collected débris. The increase in amount of lymphoid tissue as age advances is due to the irritation produced by inhaled particles.

Nerves

Parasympathetic preganglionic fibres from the vagus and post-ganglionic sympathetic fibres from the thoracic sympathetic ganglia form the pulmonary plexus at the root of the lung; this provides fibres that form plexuses in the walls of the bronchial tubes and blood vessels. In the walls of the larger bronchi are groups of ganglion cells (para-sympathetic). The parasympathetic fibres of the vagus are broncho-constrictor; the sympathetic fibres are broncho-dilator. Nerve endings, for the most part free, are found in the bronchial epithelium and in the alveolar walls: these are probably receptor in nature. Efferent parasympathetic fibres supply the bronchial glands and the muscle of the bronchial tubes whilst sympathetic fibres supply the blood vessels.

The Pleura

The pleura is the serous membrane whose parietal portion lines the pleural cavities: the visceral portion is reflected over the surface of the lung to which it is closely adherent. It consists of a thin layer of collagenous connective tissue containing fibrocytes and macrophages and a network of elastic fibres. It is covered by a layer of flat mesothelial cells, and contains very dense plexuses of blood capillaries and of lymphatics. Delicate septa pass from the pleura between the lung lobules, and the deep elastic fibres are continuous with those of the underlying alveoli. The pleural lymphatics are surrounded by a network of blood vessels derived from the bronchial artery. Nerve fibres are present and receptor nerve endings have been described.

Development of the Lungs (Fig. 18.15)

The usually accepted view is that the respiratory system develops in the same way as a secreting gland, the bronchial tubes representing the duct system and the alveoli the secreting

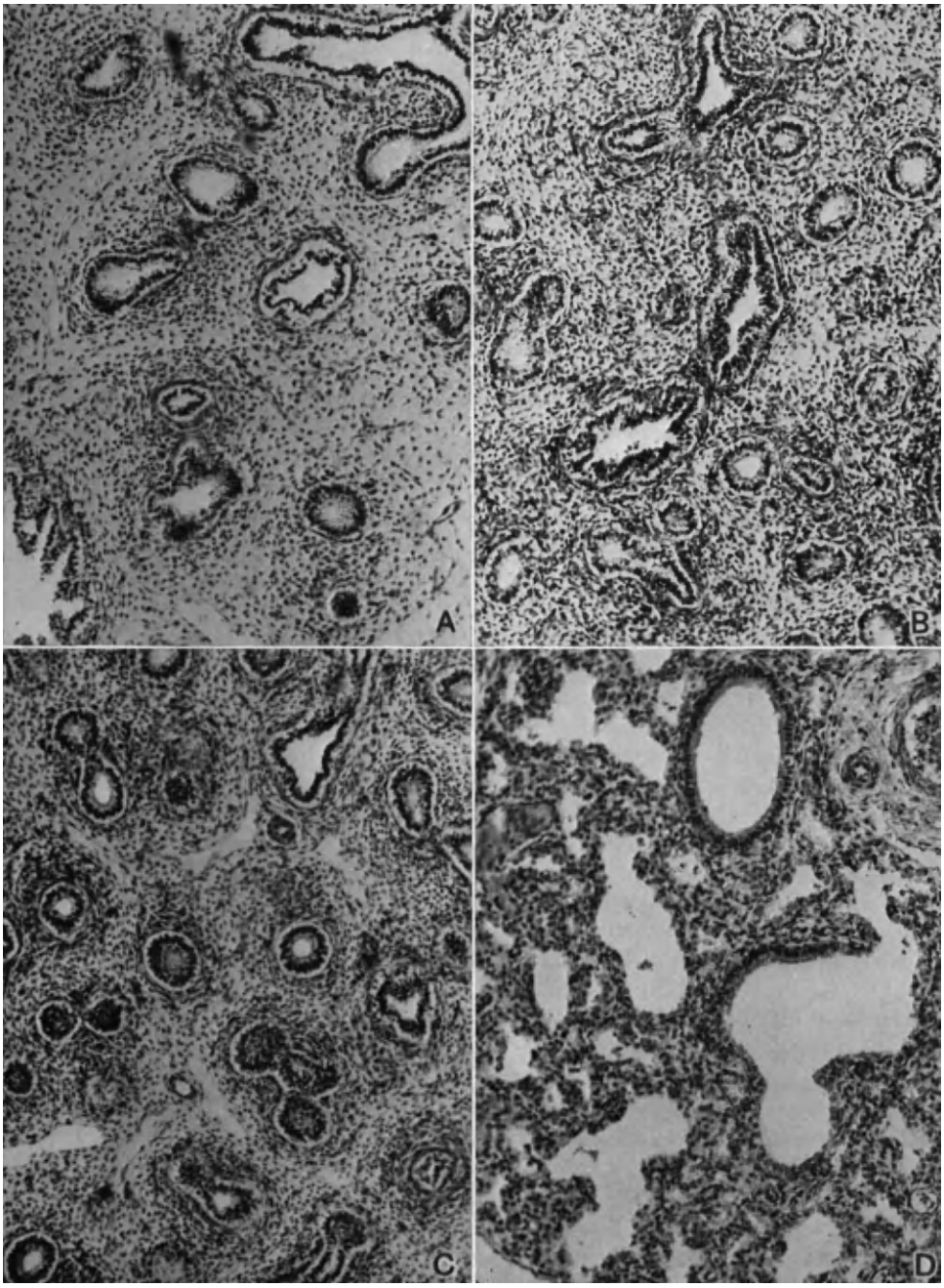

FIG. 18.15.—Photomicrographs of sections of developing human lung at different stages. A, 6 weeks; B, 13 weeks; C, 18 weeks; D, full term.

100 μm.

system, and that the foetal alveoli are lined by a cubical epithelium which becomes flattened out when the lungs are distended with air. Recent work indicates that probably the epithelium is confined to the lining of the bronchial tubes and that the origin of the alveolar sacs and alveoli is from the mesenchyme: this would account for the phagocytic potentiality of the cells that are present in the alveolar wall.

The elastic components of the lung tissue are first differentiated about the twenty-fourth week of development; by twenty-eight weeks, when the foetus becomes viable, the elastic fibres are as well developed as at birth. About the twenty-fourth foetal week also the lung loses its gland-like appearance and becomes highly vascular.

Variations in the Respiratory Organs under Different Conditions

(1) Changes during Respiration

When the body is at rest only about one-twentieth of the total alveolar surface is in use, leaving a large margin of reserve for extra need. Normally the pressure within the lungs is atmospheric, and that in the potential pleural space less: consequently the elastic lungs are kept distended. In inspiration the size of the thorax increases and the lungs are stretched still more. This increase in volume is due chiefly to a distension of the alveolar ducts and small bronchioles and bronchi, the continuity of elastic and reticular fibres between these and the blood vessel walls aiding in increasing the amount of blood flow. In expiration the elastic recoil returns the structures to their original condition.

(2) Response to Presence of Dust and other Foreign Particles

In town dwellers carbon particles are freely inhaled. Much is entangled in the mucus of the tubes and returned by the action of the cilia to the exterior. Some of it is picked up by the "dust cells" the majority of which are eventually expelled by the cough reflex and then swallowed, and some is collected into the lymphoid tissue of the lung and peri-bronchial tissue: the presence of carbon particles is very mildly irritating, but any particles of silica inhaled with them are very irritant, and the lymphoid tissue increases in amount. In coal miners the lymph nodes are usually black for this reason.

Stone particles that may be inhaled have an irritative effect and produce a fibrous response: the pleural layers may become thick and adherent, and nodules of dense fibrous tissue containing gritty particles are scattered through the lung tissue (silicosis); inhaled asbestos fibres cause a similar reaction. Extreme fibrosis usually leads to circulatory disturbances.

(3) Response to Circulatory Disturbances

Circulatory disturbances, particularly any affecting the heart, may produce profound reactive changes in the lungs. For example, any condition leading to defective output from the left side of the heart causes chronic excess of blood in the pulmonary vessels: the lung capillaries become dilated and varicose, and red blood corpuscles frequently escape into the alveoli; these cells are dealt with by the phagocytes and the pigment is carried into the dilated lymphatics.

(4) Pneumonia

The term pneumonia is applied to the inflammatory condition of the lungs resulting from some infection.

(5) Pneumothorax

When the pleural cavity becomes connected with the outside air, either from within the lungs or from outside the body (by trauma, operation or destructive lesions), the "negative" pressure that normally keeps the lung distended disappears and the lung collapses. If no

infection follows, the breach is closed and the air in the pleural cavity is gradually absorbed, the lung remaining collapsed until a "negative" pressure again develops. If infection occurs pleurisy and suppuration follow.

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CHAPTER 19

THE DIGESTIVE SYSTEM (I)

THE MOUTH

The digestive system consists of the alimentary tract, a tube beginning at the lips and ending at the anus, and of various associated structures, namely, the teeth, the tongue and the digestive glands. The organs are described in the following order:

(1) Mouth, including lips, teeth, tongue, tonsils and salivary glands.
(2) Pharynx.
(3) Oesophagus.
(4) Stomach.
(5) Small intestine.
(6) Large intestine, including colon, appendix, rectum and anal canal.
(7) Associated glands, namely, pancreas and liver.

The whole of the alimentary tract is lined by a *mucous coat* or *mucosa* which is concerned with the two important functions of *secretion* of digestive enzymes and *absorption* of the resultant products of digestion; the structure of the mucosa varies considerably in different parts: the epithelial lining is stratified from the lips to the junction of the oesophagus with the stomach, and in the anal canal, while in all other parts it is columnar in type.

Lymphoid tissue is found as definite nodules in the mucous coat, particularly in those regions where temporary stasis of the intestinal contents may occur, e.g. at the back of the mouth, at the cardiac and pyloric sphincters, in the ileum, the large intestine and the rectum: this distribution is related to the protective nature of this tissue.

Glands providing digestive juices are found in the mucous membrane of the stomach and small intestine: as the oesophagus is merely for transport it secretes only mucin. The distribution of the mucin-secreting cells is chiefly related to the need for lubricating the passage of the food mass but mucin also has a secondary role in protecting the epithelium of the gut from enzymic and chemical damage. Mucin is supplied in the mouth and the oesophagus, and also in the stomach where the food remains for some time: in the intestine the mucin-secreting cells increase in number from above downwards, and as absorption proceeds and the food mass becomes more and more of faecal consistency, the mucous cells of the epithelium far outnumber all others.

The composition of the various layers of the alimentary tract is considered on p. 292.

A. The Mouth

The oral cavity is lined by a stratified non-keratinized squamous epithelium, whose surface cells are not so flattened as those of the epidermis: they are continually being shed into the saliva. The underlying connective tissue stroma consists of delicate collagen and elastic fibres, and often projects as papillae into the overlying smooth epithelium. The arrangement of the blood vessels and lymphatics is like that of the skin. The mucous membrane is very sensitive and sensory nerve plexuses derived from the trigeminal nerve are present in the submucosa; these provide branches that form a subepithelial plexus which gives rise to fine fibres that penetrate between the epithelial cells, and also to the fibres ending in special corpuscles and taste buds.

The *lip* is covered externally by skin and internally by the buccal mucous membrane. At the junction (red margin of lip) the epithelium is much thickened, the stratum corneum of the skin passes over into the non-keratinized buccal epithelium (Fig. 19.1), and the stratum lucidum and stratum granulosum of the skin end. The connective tissue below the buccal mucous membrane contains many mucous glands opening into the mouth. In the new-born, the epithelium is considerably thickened and contains many sebaceous glands; this modification is associated with sucking.

The *gums* are covered by a thin stratified epithelium, so that the vascular stroma is near the surface: this accounts for the ease with which the gums bleed. The subepithelial papillae

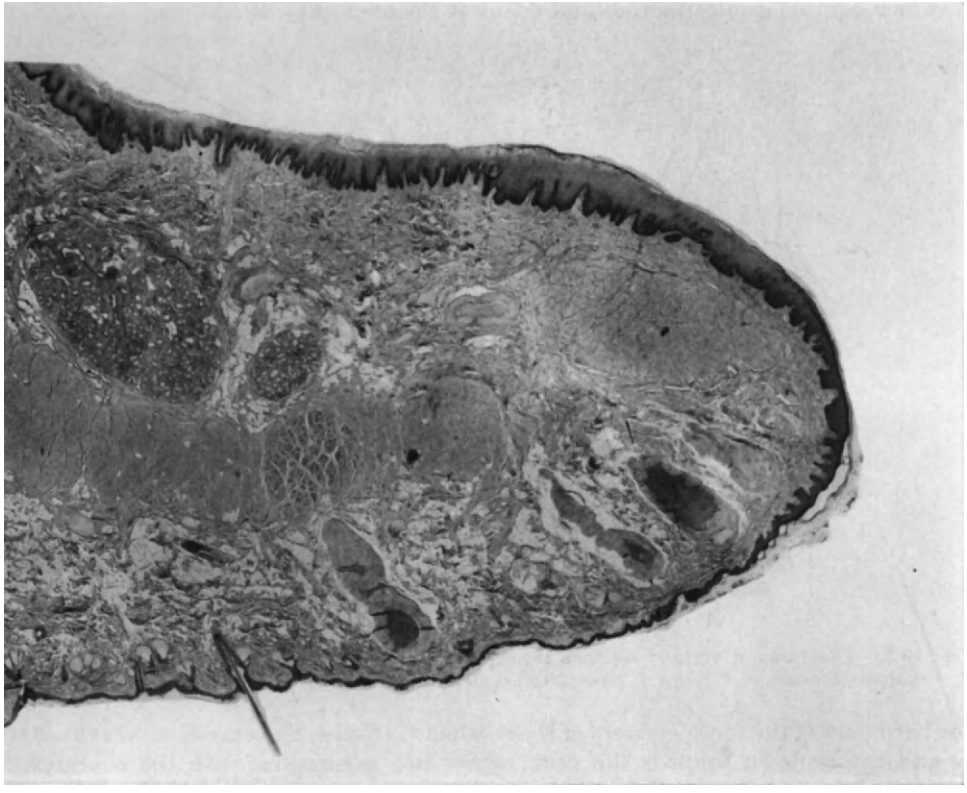

FIG. 19.1.—A low power photograph through a section of the lip of a monkey. The transition from the buccal stratified epithelium (on the upper surface) to the keratinized skin is very marked. Hair follicles (on the under surface), labial glands (upper left) and fibres of the orbicularis ori muscle (centre) may also be seen. 1·5 mm.

are richly innervated, and fine nerve fibres also penetrate into the epithelium. The stroma is attached to the underlying periosteum.

The *cheeks* are lined by a mucous membrane that has small papillae; beneath the stroma is a loose submucous layer of adipose tissue containing much elastic tissue continuous with that binding together the skin of the cheek, the fascia, the muscles and the mucous membrane. This arrangement provides the cheeks with a considerable degree of extensibility and elasticity. The stroma contains many glands opening into the mouth.

The *hard palate* has no submucosa, but the stroma of the mucous membrane is connected with the underlying periosteum or striated muscles. The papillae are short, and the glands of pure mucous type.

Over the *soft palate* the papillae are often absent: on the *uvula* the papillae are usually long and the stratified squamous epithelium is interrupted by areas of columnar ciliated pseudo-stratified epithelium. The mucous membrane of the soft palate, uvula and fauces contains diffuse lymphoid tissue, often aggregated into nodules.

B. The Teeth

A tooth can be regarded as a modified papilla, developed partly from epithelium and partly from underlying connective tissue (see p. 277). It consists of a *crown*, which projects above the gum, and a *root*, which tapers down to fit tightly into an excavation of the bone of the jaw, the *alveolus*. Where the crown and the root meet is called the *neck*. The tooth contains a *pulp cavity*, which continues into the root and opens at the apex (Fig. 19.2).

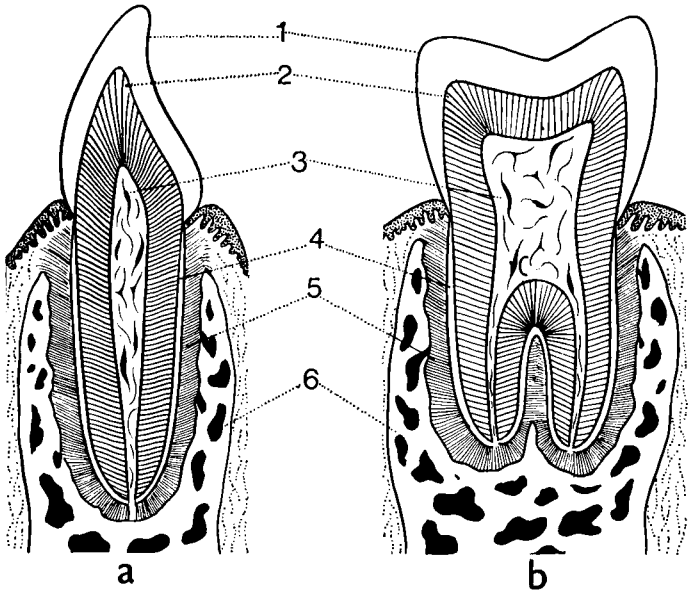

FIG. 19.2.—Diagrams of vertical sections through an incisor tooth (*a*) and a molar tooth (*b*). 1, enamel; 2, dentine; 3, pulp; 4, cementum; 5, dental periosteum; 6, bone forming the alveolus.

The hard part of the tooth consists of three calcified tissues. The *enamel* covers the exposed crown and tapers down towards the neck, where it is continuous with the *cementum*: the cement covers the buried part of the tooth except at the apex, where the pulp canal opens. The main bulk of the tooth consists of *dentine*, which surrounds the pulp cavity.

Enamel

Tooth enamel is the hardest substance in the body: 96 per cent of the enamel consists of inorganic salts, and as calcium phosphate and carbonate form the major part of this the enamel has been usually completely removed in decalcified preparations.

Enamel consists of six-sided prisms, about 5 μm. in diameter, standing upright on the outer surface of the dentine with an inclination towards the crown: a small amount of cement substance binds them together. Lines of cross striation can sometimes be seen, and this probably indicates calcification by layers.

Dentine

Dentine is similar to bone, but harder, and contains no lacunae or bone cells. It consists of innumerable canaliculi (*dentinal tubules*) diverging from the pulp cavity and lying in a calcified

ground substance that contains collagen bundles running parallel to the surface of the pulp: these represent the lines of increment of the dentine. The dentinal tubules are about 2 μm. in diameter at their origin, but become narrower and give off branches as they pass outwards. The dentine immediately surrounding a canal is harder and denser than elsewhere, and is known as the *dentinal* (*Neumann's*) *sheath*. Each dentinal tubule contains a protoplasmic fibre (*fibre of Tomes*) which is a process of an odontoblast: these cells are arranged on the wall of the pulp cavity.

Cementum

The cementum or cement covering the dentine of the root is coarse-fibred bone, containing bone cells particularly near the apex; definite Haversian systems are usually absent. Its ground substance is continuous with that of the dentine. The cement is covered by the *dental periosteum* (or periodontal membrane), which also lines the alveolus: the fibres of this periosteum penetrate into both cement and bony wall and so fix the tooth securely.

Pulp

The pulp fills the pulp cavity. It consists of the vascular connective tissue of the original dental papilla. The ground substance is soft, gelatinous and basiphilic: its fine collagen fibres run in every direction and not in bundles, and the cells are largely spindle-shaped or stellate. Macrophages are also present and the general appearance is that of embryonic connective tissue. The peripheral cells are grouped like an epithelium and are the large, long *odontoblasts*, whose processes pass into the dentinal tubules.

Blood Vessels

The arteries enter the dental periosteum and divide into two branches, one passing outside the tooth to supply the dental periosteum and the other entering at the apex to supply the pulp. In the pulp the vessels run chiefly through the middle, giving off branches that make a capillary network that is specially dense at the periphery. The veins pass back through the apical foramen to join the veins returning from the periosteum. The enamel, cement and dentine have no blood vessels.

Lymphatics

Lymphatic capillaries have been seen in the pulp of the crown, the larger lymphatics issuing with the blood vessels at the apex.

Nerves

The distribution of nerve fibres follows that of the blood vessels. Myelinated fibres issuing from the apex are dendrites of cells in the trigeminal (Gasserian) ganglion. They have formed primary and secondary plexuses in the pulp. Sensory nerve endings have been described lying between the odontoblasts, and between the odontoblasts and the dentine. The sensitiveness of the dentine, in spite of the apparent absence of nerve fibres, may be due to a stimulus being carried to the nerve endings through the processes of the odontoblasts.

Development of the Teeth (Fig. 19.3)

The enamel of the tooth is of ectodermal origin, all the remainder being mesodermal. The first indication of the formation of the teeth is the appearance at about the fifth foetal week of a thickening of the epithelium covering the edge of the jaw and dipping into the underlying mesoderm to form the *common dental lamina* (Fig. 19.4). At regular intervals along the outer side of the lamina further thickenings appear, ten in each jaw, extending deeper into the connective tissue: these swell out into the *special tooth germs* of the milk teeth. As development proceeds the connection between the tooth germ and the dental lamina becomes constricted

into a thin stalk. From about the twelfth foetal week onwards the dental lamina produces more local thickenings, one on the lingual side of each of the original germs. These are the permanent tooth germs, and after their formation the dental lamina disappears. The further development of milk and permanent teeth is the same.

Immediately beneath and to one side of the epithelial downgrowth the adjacent connective tissue becomes condensed as the primordium of the *dental papilla*. This tissue invaginates into

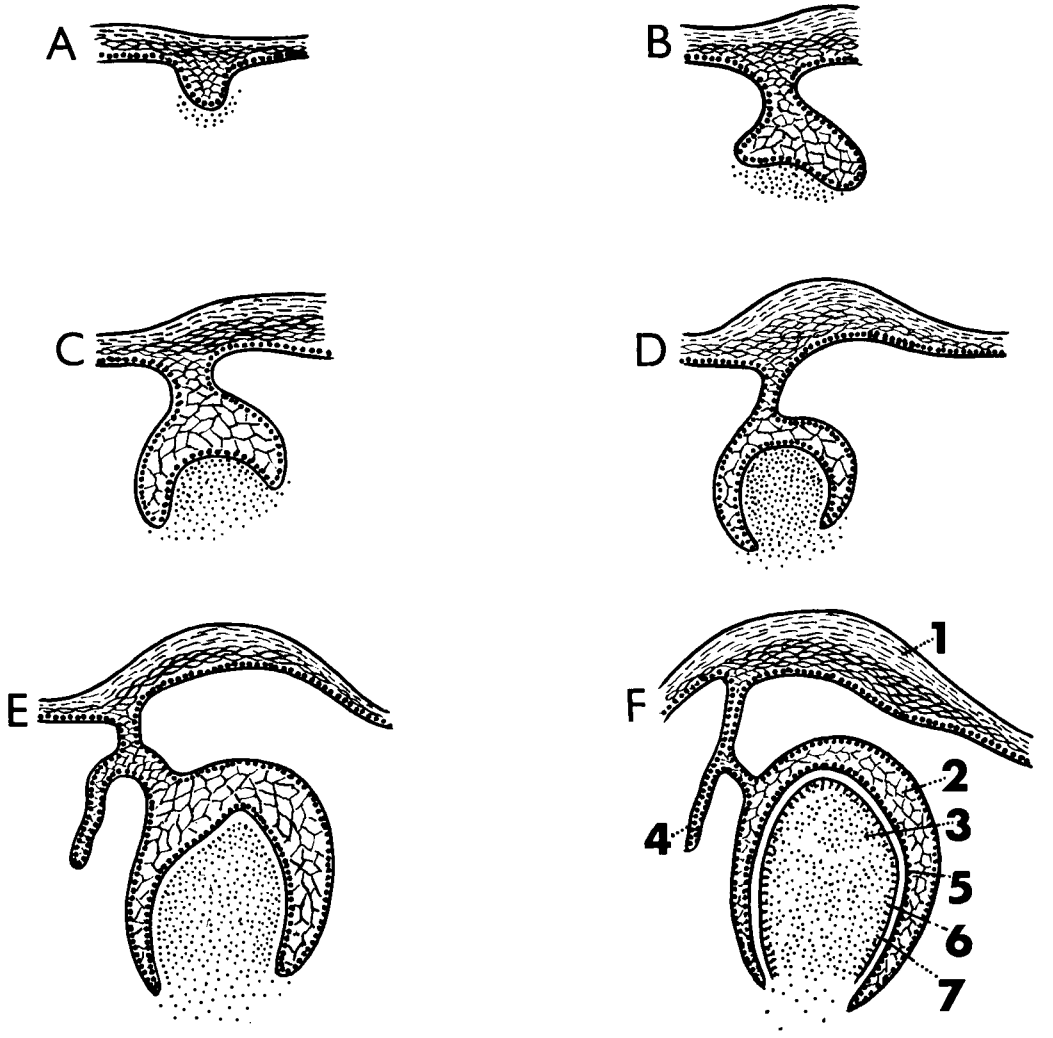

FIG. 19.3A–F.—Diagrams of various stages in the development of a tooth. 1, epithelium of the gum; 2, enamel organ; 3, dental papilla; 4, enamel organ of permanent tooth on lingual side of dental papilla; 5, ameloblast layer; 6, odontoblast layer; 7, dentine.

the dental germ, so that the latter fits like a cap over the papilla. The dental germ will give rise to the enamel and is known at this stage as the *enamel organ*: the vascular dental papilla will give rise to the dentine and the pulp (see Figs. 19.5, 19.6).

The papilla and the enamel organ continue to grow, and in so doing stretch the elements of the surrounding tissue so that a layer of concentrically-arranged connective tissue comes to

surround the developing tooth: this is the *dental sac*. The epithelial connection with the surface ultimately disappears. The bone of the jaw develops round the dental sac some distance away. The enamel organ then differentiates into three layers, the outer flattened cells, the large mass of enamel pulp consisting of stellate anastomosing cells with much intercellular substance, and the inner layer of high columnar *ameloblasts* (Fig. 19.6). The ameloblasts form first a fibrous membrane and then the enamel prisms on their free surface, i.e. next to the dentine. The enamel pulp and external enamel cells disappear. Although the enamel organ surrounds both root and crown, enamel is formed only over the crown.

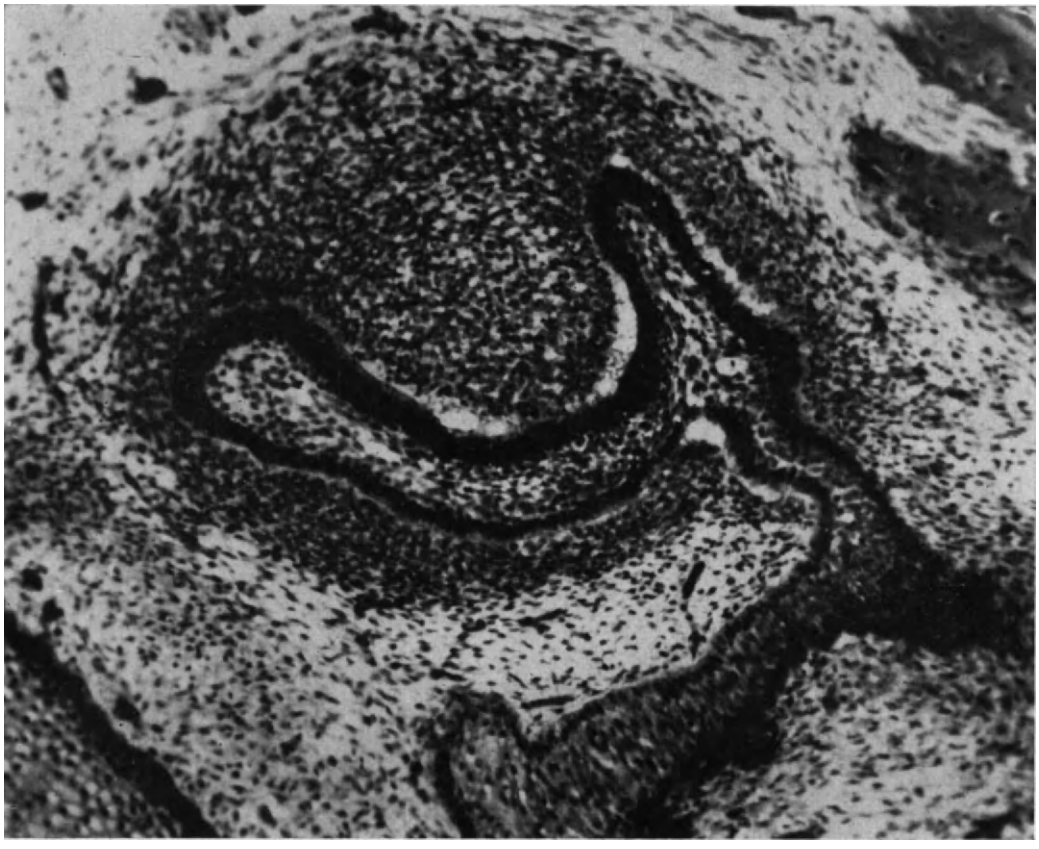

FIG. 19.4.—A section through a developing tooth in a human embryo. The enamel organ surmounting the dental papilla (which is still connected to the surface by epithelial cells of the dental lamina) is clearly shown. 80 μm.

The dentine is formed over the surface of the papilla by the *odontoblasts* (Fig. 19.6), which form the external layer of the pulp and consist of irregularly columnar cells. Processes of the odontoblasts appear to remain in the dentine as it forms and so produce the dentinal tubules. In this way the dentine comes to lie next to the enamel. The coat of dentine is increased in thickness by the formation of another layer within the first, the process being repeated many times: some part of the papilla however always remains uncalcified.

The *root* of the tooth begins to form shortly before its irruption. As the root grows in length it meets the bone of the jaw, so that the tooth gets pushed towards the surface; the pressure at the surface causes atrophy and the crown of the tooth pierces the gum. When the germ of the permanent tooth begins to develop under the milk tooth the pressure causes resorption of

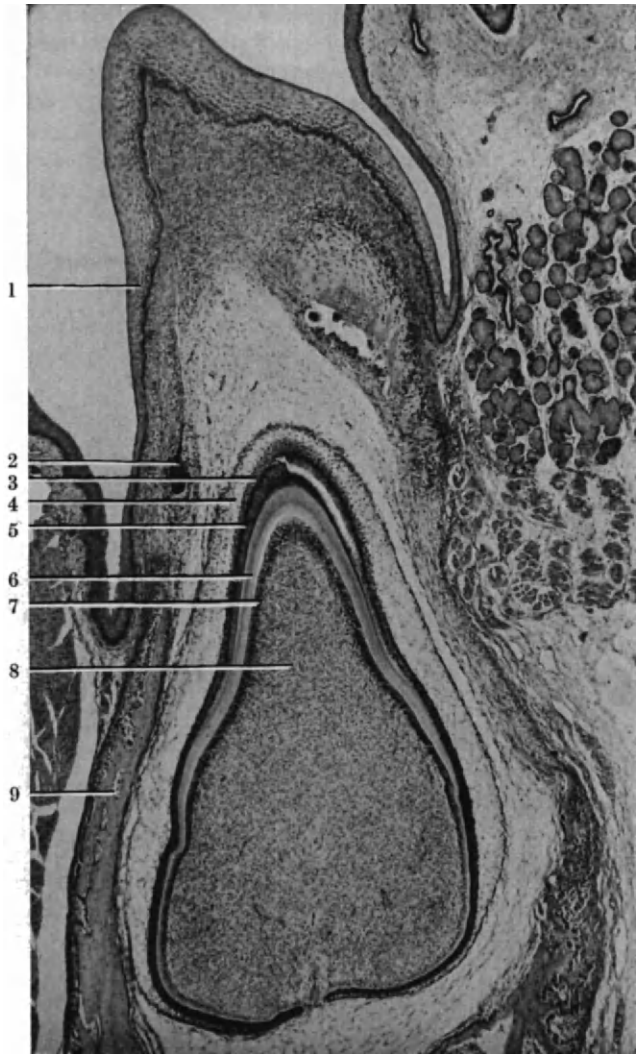

FIG. 19.5.—A micrograph through a section of a tooth in a human foetus. 1, epithelium of gum; 2, permanent tooth germ; 3, ameloblasts; 4, enamel organ; 5, enamel; 6, dentine; 7, odontoblasts; 8, pulp of tooth; 9, bone of jaw.

the root and the milk tooth becomes loose in its socket and then falls out: this resorption is largely due to the activity of osteoclasts (see p. 105).

C. The Tongue

The tongue consists of striated muscle covered by a mucous membrane. The muscle is arranged in bundles, closely interwoven, running in the vertical, transverse and longitudinal directions, and crossing one another at right angles (Fig. 19.7). The bundles are bound together by connective tissue which contains some fat and some mucous and serous glands. A particularly strong band of connective tissue extends through the length of the tongue dividing it into halves.

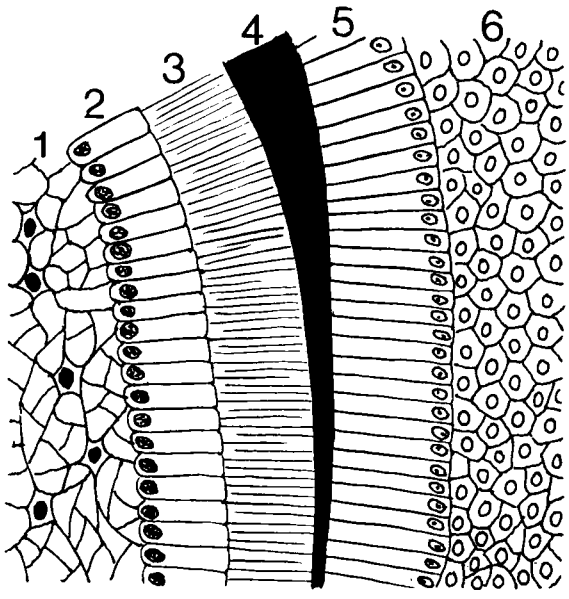

FIG. 19.6.—A diagram through a developing tooth. 1, pulp of tooth; 2, odontoblasts; 3, dentine; 4, enamel; 5, ameloblasts; 6, enamel organ.

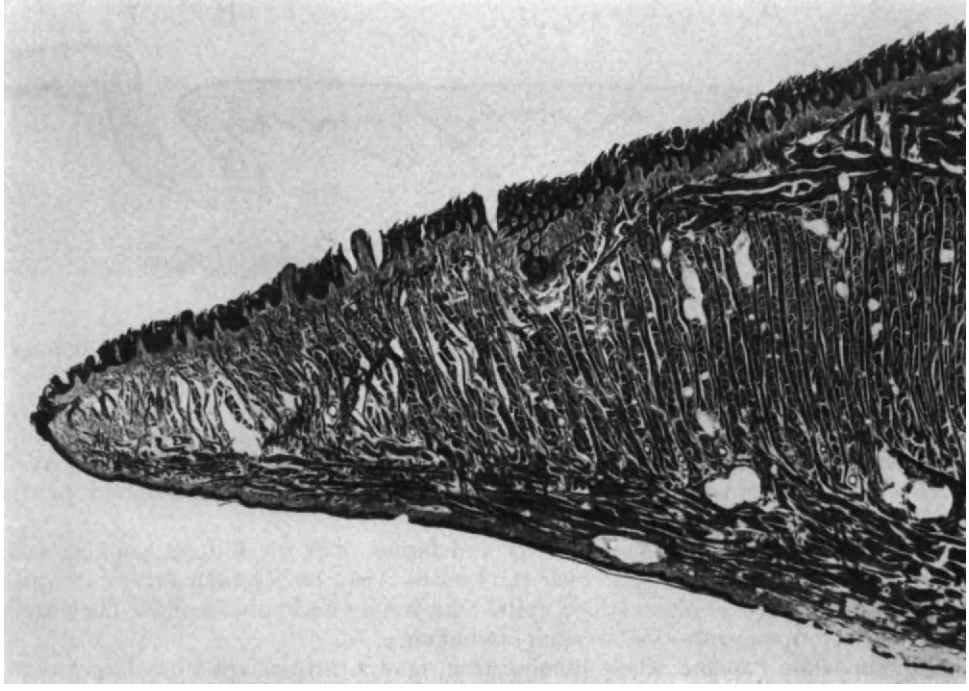

FIG. 19.7.—A low power micrograph of the tongue in longitudinal section. The papillae on the upper surface may be clearly seen. Note the numerous muscle fibre bundles, running in several planes.

1·5 mm.

The *mucous membrane* consists of a thick stratified epithelium and an underlying corium of connective tissue containing many blood vessels, lymphatics and nerve fibres. The under surface of the tongue is smooth, but the upper surface is covered with excrescences of varying form and size, consisting of definite papillae in the anterior part and nodules of lymphoid tissue in the posterior part. The boundary between the anterior and posterior regions is V-shaped, with the angle directed backwards.

Three kinds of *lingual papillae* are distinguished (Fig. 19.8): within each the connective tissue core may give rise to secondary papillae.

(1) **Filiform Papillae.** These are the most numerous, and are distributed over the whole surface. Each papilla has a thin core of connective tissue and is covered by a pointed cap of stratified epithelium which is cornified and often drawn out into projecting threads. Normally

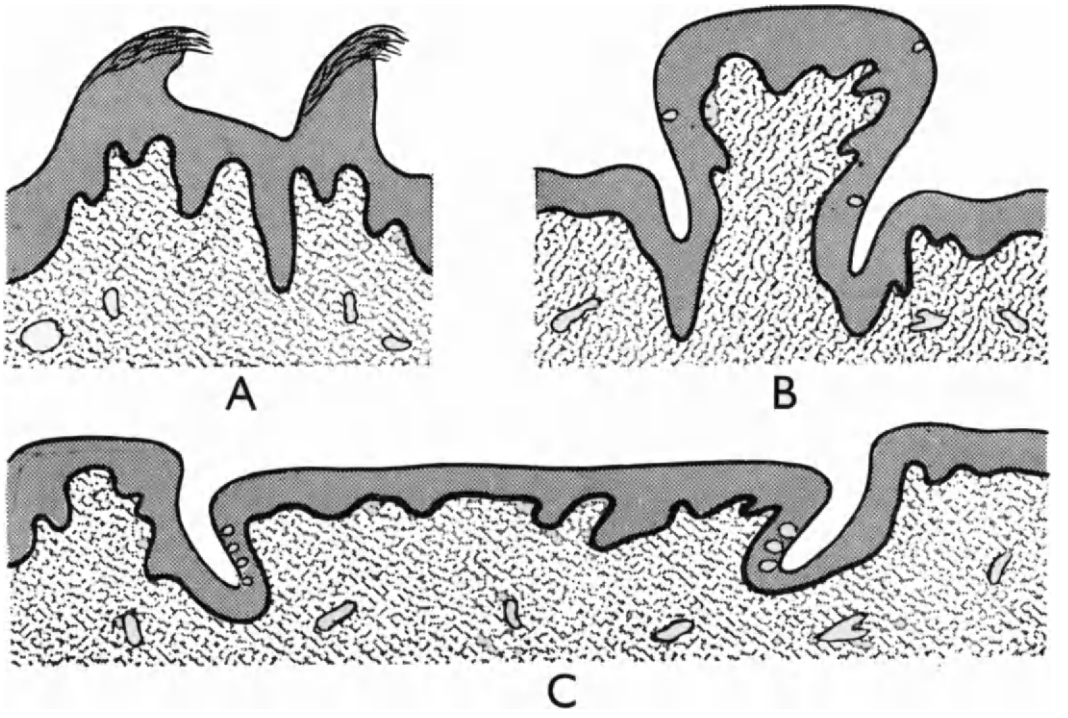

FIG. 19.8.—A diagram of the various forms of papillae on the human tongue. A, filiform; B, fungiform; C, circumvallate. Taste buds occur on the two latter types.

these scales are shed continually: if digestion is disturbed, the shedding is delayed and the scales accumulate mixed with bacteria on the surface, giving the characteristic "coated" or "furred" tongue. (In many animals—e.g. cat—these papillae are hard and curved, producing a scraping effect when the animal licks.)

(2) **Fungiform Papillae.** These are fewer and larger than the filiform papillae and are scattered irregularly among them all over the surface. They have rounded summits and narrower bases, and the covering stratified epithelium is thin and not cornified. They are very vascular and have some taste buds in their epithelium.

(3) **Circumvallate Papillae.** These number from nine to fifteen, are much larger than the fungiform papillae, and are arranged along the V-shaped division between the anterior and posterior parts of the tongue. Each papilla is sunk into the mucous membrane and is surrounded by a deep invagination of the surface epithelium. Lying in the stratified epithelial wall of

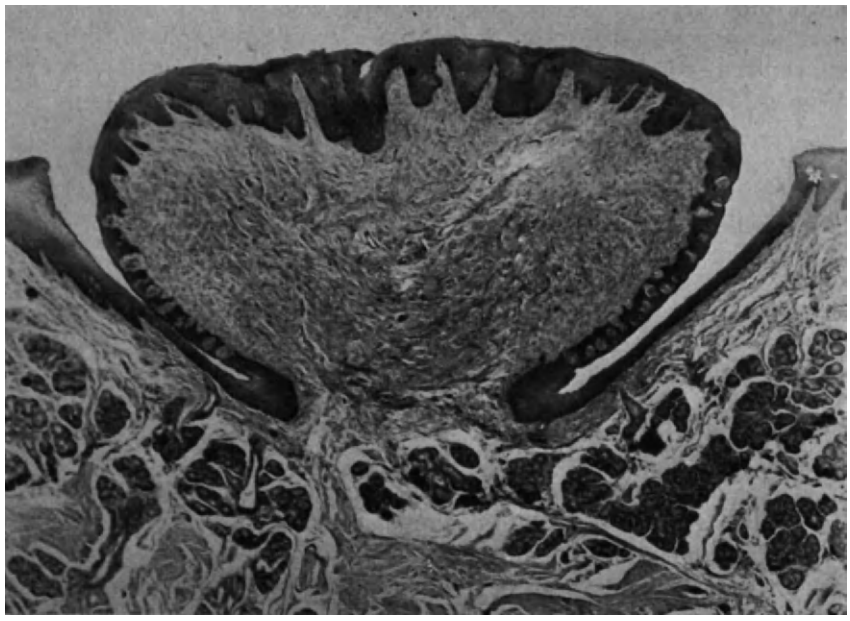

FIG. 19.9.—A photomicrograph of a circumvallate papilla from the tongue of a monkey. The stratified epithelium and the taste buds at the side of the papilla are visible, as well as alveoli of serous glands (Ebner's glands).

50 μm.

the trench are many taste buds. The ducts of serous glands (Ebner's) open into the trench (see Fig. 19.9).

Taste Buds (Fig. 19.10)

A taste bud is shaped rather like a barrel standing on its end: it extends through the thickness of the stratified epithelium to open on the surface by a minute hole, the gustatory pore. Two types of cells are present:

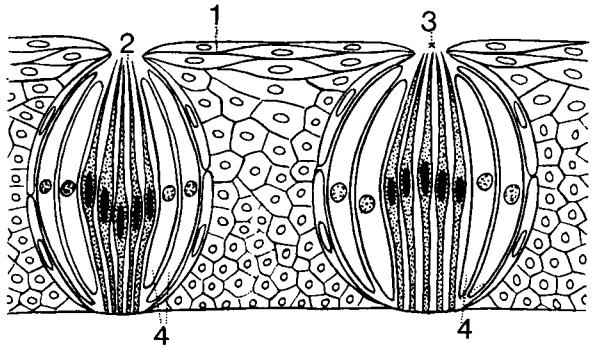

FIG. 19.10.—A diagram of the organization of a taste bud. 1, stratified epithelium of the tongue; 2, gustatory cells; 3, gustatory pore; 4, sustentacular cells.

(1) **Gustatory Cells.** These are slender bipolar cells with a central enlargement for the nucleus and tapering at both ends: the distal process passes towards the gustatory pore and ends in a highly-refractile hairlet. The proximal process is fine and branched. Sensory (intragemmal) nerve fibres arise among these cells, whilst others (perigemmal) ramify around the taste bud itself. It is also reported that some intergemmal fibres arborize between adjacent taste buds.

(2) **Sustentacular Cells.** These are long, crescentic cells, pointed at the ends, and with broad oval nuclei supporting the gustatory cells and forming the walls of the taste bud.

(In some animals—e.g. rabbit—the taste buds are grouped in large numbers in the easily recognized foliate papillae. This provides convenient material for their study.)

Blood Vessels

The tongue is very vascular. The muscle fibres have the usual capillary network, and a vascular plexus beneath the epithelium gives off branches to all the papillae.

Lymphatics

Lymph spaces in the papillae open into a plexus of lymph capillaries in the deeper connective tissue layers. These drain into a deeper plexus of lymphatics, which is particularly well developed in the region of the lingual tonsils at the root of the tongue.

Nerves

The blood vessels and glands are supplied by sympathetic fibres. The striated muscle of the tongue is richly supplied with myelinated motor nerve fibres arising from the XIIth cranial (hypoglossal) nerve. The sensory nerve endings include the free nerve endings among the surface epithelial cells, and those from the encapsulated taste buds chiefly in the circumvallate papillae; Krause's end bulbs have been found in the fungiform papillae. These nerve fibres run back to the CNS via the lingual nerve and the chorda tympani nerve, together with some in the glossopharyngeal nerve.

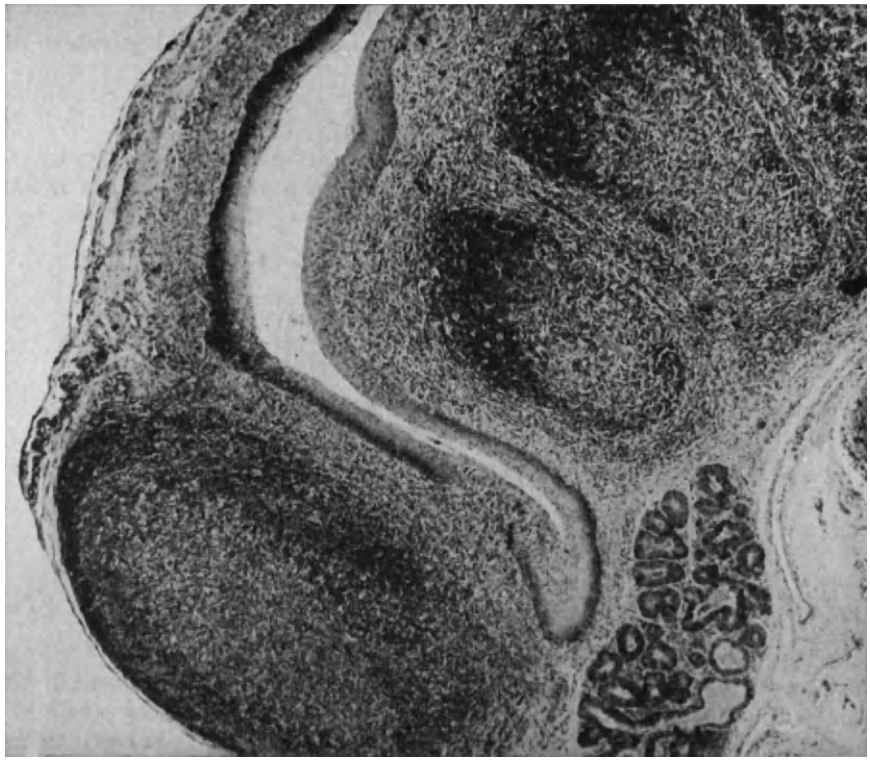

FIG. 19.11.—A section through the tonsil of a cat. The space in the centre of the picture lined by stratified epithelium, is a section through part of a crypt. 150 μm.

Tonsils (Figs. 19.11, 19.12)

In the mucous membrane of the root of the tongue and of the neighbourhood of the fauces there are found accumulations of lymphoid tissue invaginated by the surface stratified epithelium: these are easily seen as well-defined organs, the *lingual and palatine tonsils* and the median *naso-pharyngeal* tonsil in the posterior wall of the naso-pharynx.

The palatine tonsil is covered by stratified epithelium and the surface is pitted by numerous deep furrows or crypts. The crypts are lined by stratified epithelium and receive the openings of the ducts of many mucous glands. The substance of the tonsil surrounding the crypts consists of diffuse lymphoid tissue in which are embedded numerous lymph nodules, most of

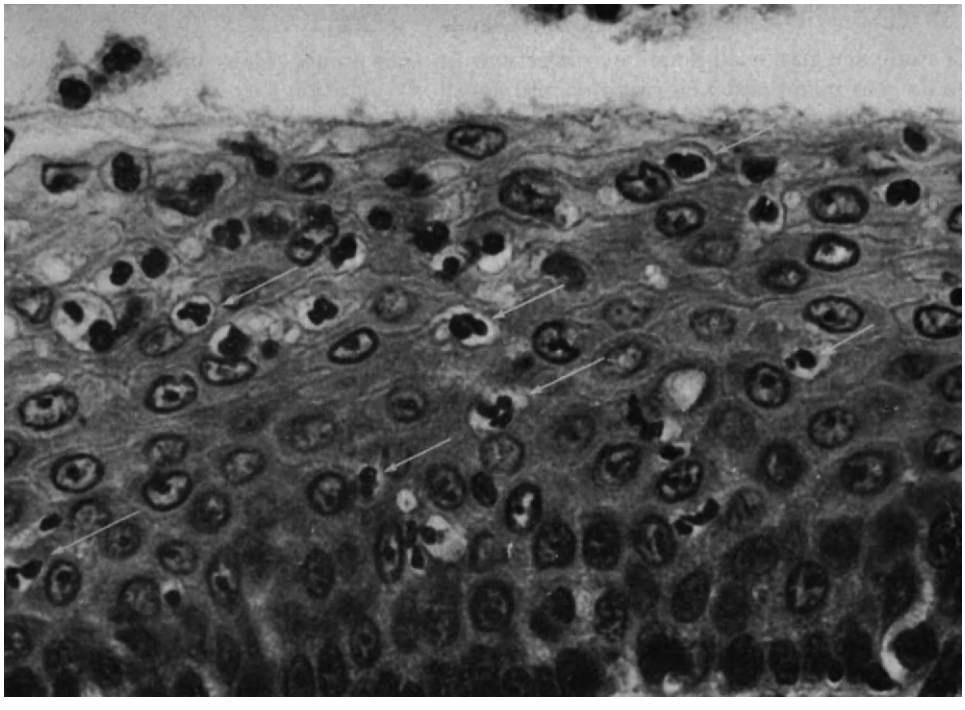

FIG. 19.12.—A high power view of the stratified epithelium covering the tonsil. Many lymphocytes and polymorphonuclear leucocytes (arrowed) have infiltrated into the epithelium. 20 μm.

them containing germinal centres in which active proliferation of lymphocytes occurs. The surface epithelium is infiltrated with lymphocytes, many of which pass out to mix with the saliva (Fig. 19.12). Thin partitions of connective tissue pass between the various masses of lymphoid tissue round the crypts, and in this connective tissue are found mast cells, plasma cells, macrophages and leucocytes: the presence of polymorphonuclear leucocytes indicates inflammation.

The lumen of a crypt usually contains many lymphocytes, some alive and many degenerating, also desquamated epithelium and micro-organisms. These micro-organisms are frequently the cause of various infections.

The pharyngeal tonsil in the naso-pharynx sometimes hypertrophies and obstructs the nasal passages: this is the condition that in children is called "adenoids".

Blood Vessels

As there is no hilum to these lymphoid masses the blood vessels enter all along the attached border and are distributed as in lymph glands.

Lymphatics

There are no afferent lymphatics or lymph sinuses and lymph does not filter through the tonsils. The lymphoid nodules are surrounded, but not penetrated, by a plexus of blindly-ending lymph capillaries by means of which the lymph is drained away.

Nerves

Nerve fibres supply the tonsil, entering usually with the blood vessels.

D. Salivary Glands (Fig. 19.13)

The salivary glands all pour their secretions into the mouth. Many of the small glands lie in the mucous membrane and secrete continuously. In addition there are three pairs of large glands (parotid, submandibular and sublingual) that lie outside the mucous membrane and are connected with the mouth by their large ducts; these secrete (in man) only in response to certain stimuli, usually mechanical or chemical or olfactory.

All the salivary glands are compound tubulo-alveolar glands: they consist of true glandular tissue and a connective tissue framework comprising capsule and trabeculae subdividing the lobules. The connective tissue contains fibrocytes, macrophages, fat cells, plasma cells and lymphocytes. The secreting alveoli may contain cells of various types, some secreting mucin and some secreting a serous fluid containing ptyalin.

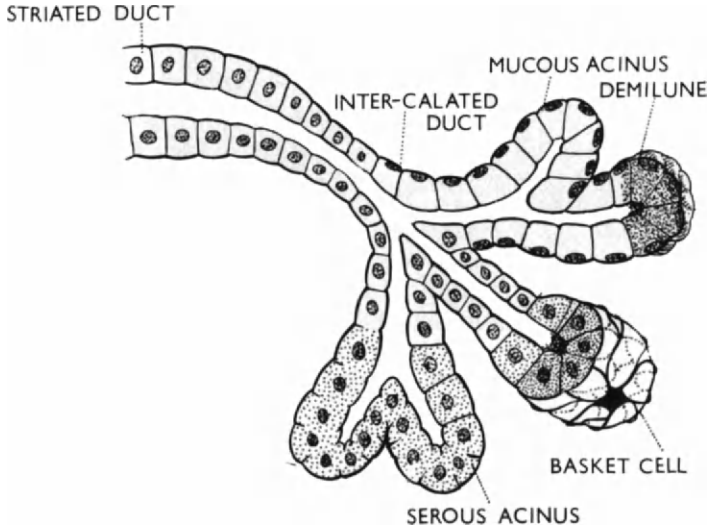

FIG. 19.13.—A diagram of the typical organization of a mixed salivary gland.

Ducts

The ducts are lined by columnar epithelium with scattered basal cells between them and the basal lamina. In the large (interlobular) ducts the cells are clear and are arranged in the form of a pseudostratified columnar epithelium: in the smaller (intralobular) ones, sometimes called the "striated" ducts, the cells are not definitely marked off from one another when examined with the optical microscope and show striations in the outer half of the cells next the basal lamina due to the arrangement of the mitochondria.

The E/M shows the presence of numerous basal infoldings of the plasma membrane of the cells of these striated ducts, an appearance resembling that found in other epithelia when active transport of water and ions is taking place (see p. 343). Histochemical and autoradiographic evidence suggests that these smaller striated ducts may be more than passive conveyors of the salivary secretions, but whether this is also likely in the human has yet to be established. The smallest part of the intralobular duct opening from the alveolar lumen is lined by flattened cells and is known as an intercalary duct. The intercalary ducts are long in the parotid gland, intermediate in length in the submandibular and extremely short in the sublingual gland.

Alveoli (Figs. 19.13, 19.14)

The alveoli are surrounded by a basal lamina continuous with that of the ducts. Within this lie the polyhedral secreting cells; they surround the central lumen that opens out into the duct. Between the basal lamina and the secreting cells are flat, branched cells, possibly modified

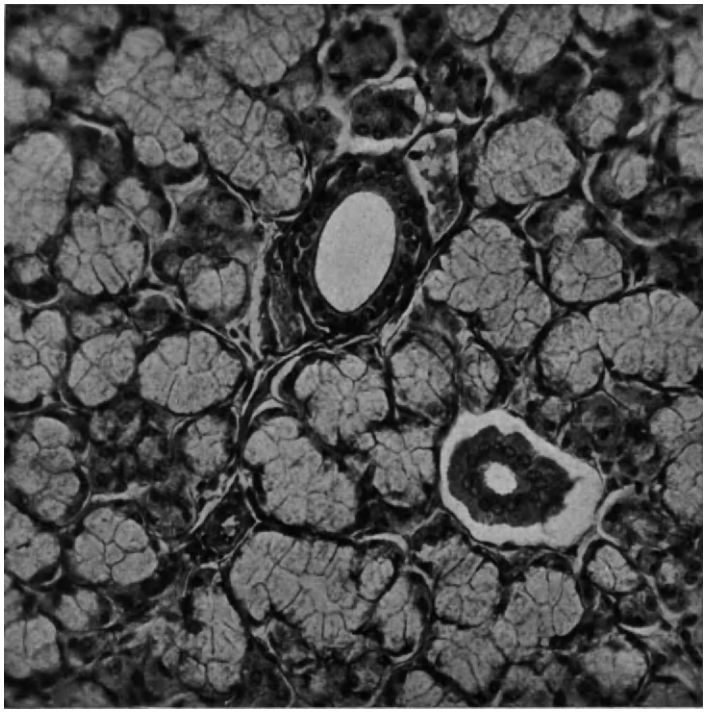

FIG. 19.14.—A section of the submandibular salivary gland of a cat. This is a mixed gland; the mucous cells are lightly staining whilst the serous cells colour more darkly. Two ducts are seen in cross section, one of which has shrunk away from the surrounding tissue during the preparation of the specimen. 50 μm.

smooth muscle: these are called "*basket cells*" (Fig. 19.15), and correspond to the myo-epithelial cells in the sweat glands (see p. 248), and mammary glands (see p. 390).

Mucous Cells (Figs. 19.14, 19.16)

The cells are large, and in the fresh condition the cytoplasm contains large droplets of mucinogen and the nucleus is invisible. When fixed the cell body looks clear, but for an (artificial) loose network of precipitated mucinogen; this gives the staining reactions for mucin. The nucleus is at the base of the cell, flattened against the basal cell membrane if the cell is full of stored secretion. In sections stained with haematoxylin and eosin, for example, the

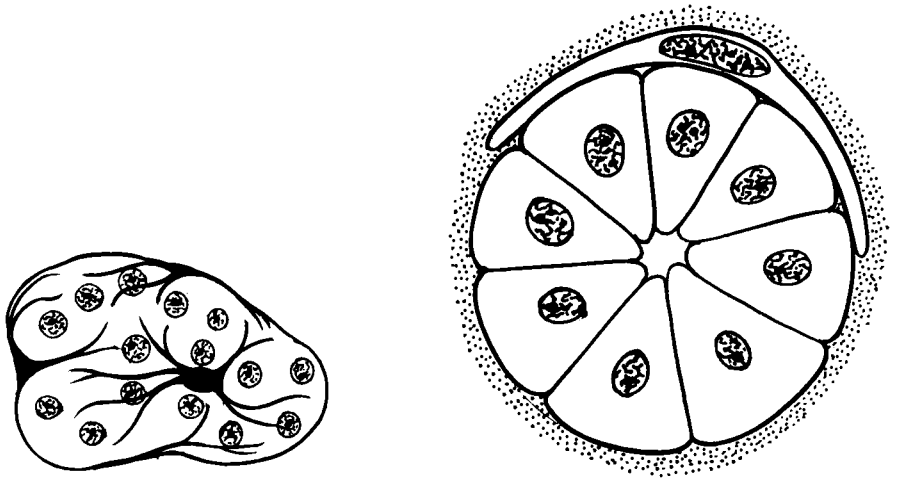

FIG. 19.15.—A diagram of the arrangement of basket cells around an acinus. On the left an acinus is seen in surface view with three basket cells (black) around it. A single acinus in section (on the right) has one basket cell; the basal lamina is shown in stipple.

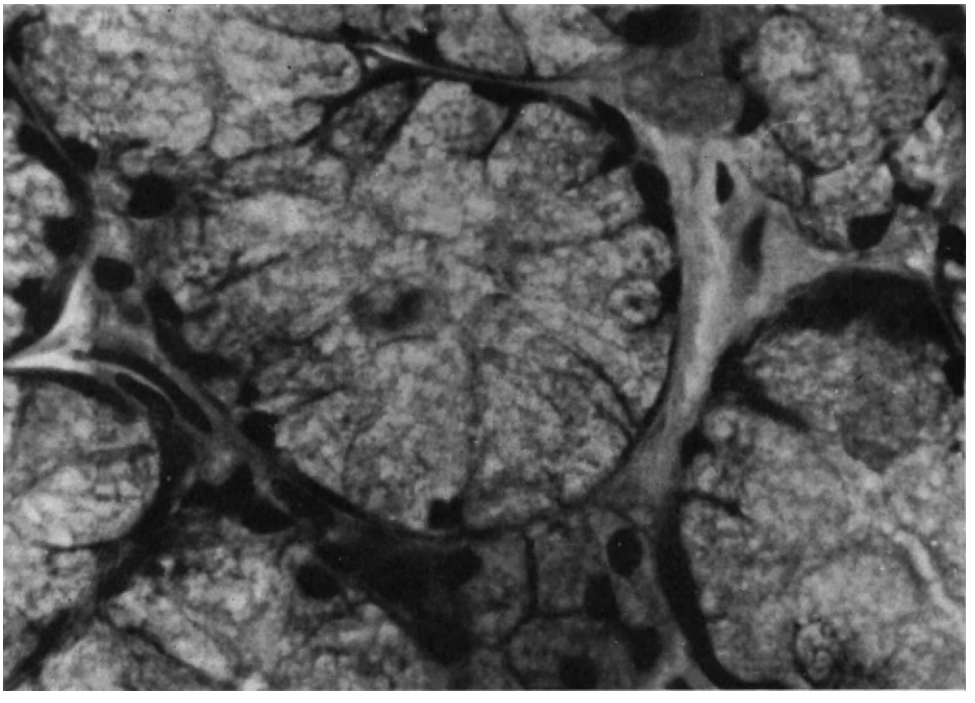

FIG. 19.16.—A photomicrograph of mucous cells from the sublingual gland. The mucus has not been stained, hence the rather "empty" appearance of the cells. The nuclei have been pushed towards the basal aspect of the cells by the accumulated secretion. 15 μm.

precipitated mucinogen often stains pink with the eosin, whereas the cytoplasm of the serous cells of the capping demilunes (if present, see below) is coloured purple with the haematoxylin. During physiological activity the cells discharge their secretion as mucin into the lumen, and the emptied cells then appear small and granular, with a round, central nucleus.

Serous Cells (Figs. 19.14, 19.17)

The cells are usually smaller than the mucous cells. In the fresh condition they contain numbers of small secretion granules which can be fixed and stained in the usual way. When the cells are resting they are filled with these darkly-staining granules, and the basal spherical nucleus is somewhat obscured by them. When actively secreting, the cells discharge the granules into the lumen, and first the outer part of the cell becomes free of granules and then the whole cell: the nucleus is then easily seen.

In mixed glands that also contain mucous alveoli, each mucous alveolus is capped at its blind end by a crescent-shaped group of serous cells. These are called the *crescents* or *demilunes* (Fig. 19.18). The secretion from these demilune cells is carried by secretory capillaries between the mucous cells into the lumen of the alveolus.

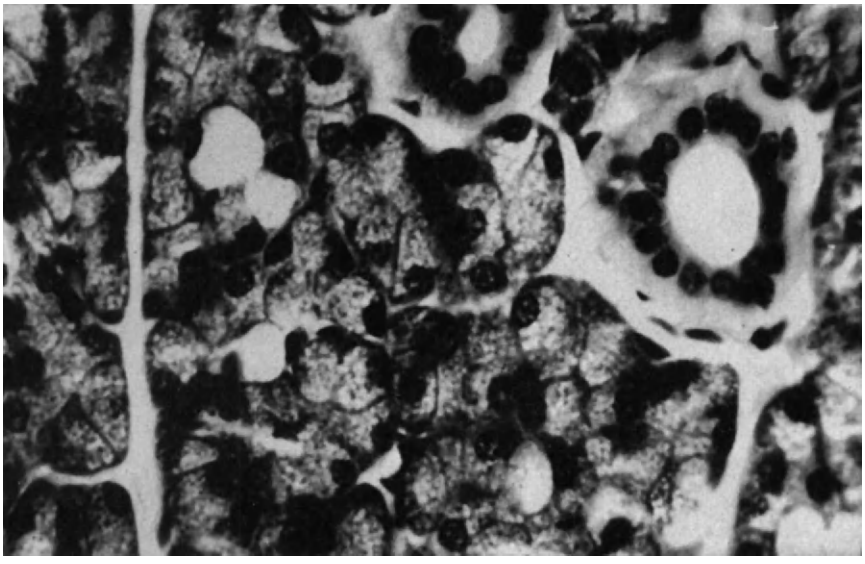

FIG. 19.17.—A micrograph of the parotid gland. Note the presence of intralobular fat cells. The duct on the right of the picture is an intralobular duct. 60 μm.

Structure of the Various Oral Glands in Man

(1) **Labial glands** in the mucous membrane of the lips, and **buccal glands** in that of the cheeks.

Mixed glands, containing serous and mucous alveoli.

(2) **Lingual glands.**

(*a*) In the anterior part of the tongue—
Mucous glands with serous demilunes (anterior lingual glands).

(*b*) In the posterior part of the tongue—
Serous glands whose ducts open into the trenches of the circumvallate papillae.
Pure mucous glands at the root of the tongue.

(3) **Palatine glands.**

Mucous glands only.

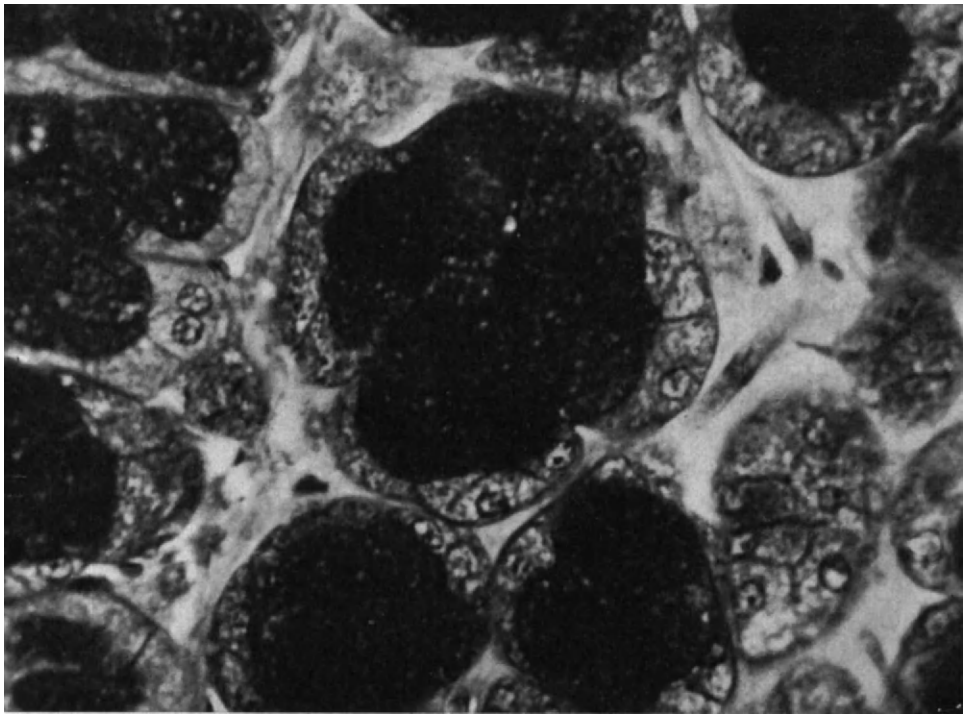

FIG. 19.18.—Mucous cells in the submandibular gland. The mucus appears dark in this preparation. Note how the mucous cells are capped by the serous demilunes which stain more lightly.

20 μm.

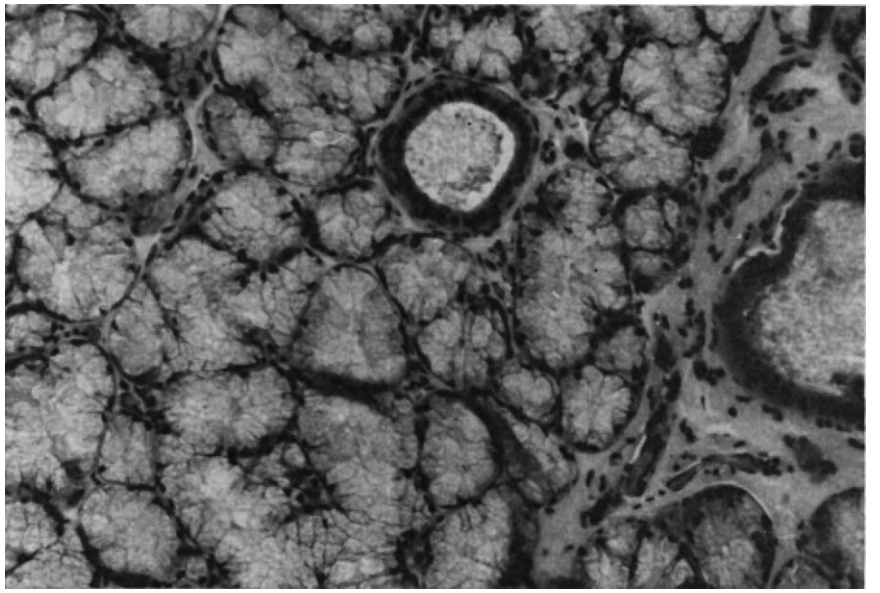

FIG. 19.19.—The appearance of the sublingual gland. Note that the bulk of the acini are mucus-secreting.

100 μm.

(4) Salivary glands.

The characteristics of the salivary glands are summed up in the following table.

	PAROTID (Fig. 19.17)	SUBMANDIBULAR (Fig. 19.14)	SUBLINGUAL (Fig. 19.19)
Secretory cell type	Serous with very occasional mucous	Mixture of serous and mucous; some demilunes present	Majority mucous; few serous demilunes
Intercalated ducts	Long	Variable	Virtually absent
Intralobular ("Striated") ducts	Long	Branched and very long	Very short
Intralobular fat cells	Numerous	Few	Virtually absent
Main duct	Single	Single	Multiple
Capsule	Present; thick	Present; average thickness	Present; very thin

Blood Vessels and Lymphatics

The larger blood vessels follow the ducts in the connective tissue septa, branching with them and providing capillary networks round the small ducts and the secreting alveoli. The lymphatics arise in the connective tissue between the alveoli and empty into larger vessels that run with the arteries.

Nerves

Each gland possesses afferent (sensory) myelinated nerve fibres, accompanying the ducts and arising in the alveoli from a sub-epithelial plexus as fibrils between the epithelial cells. Each gland also possesses efferent (secreto-motor) fibres of the parasympathetic system: the ganglion cells are in the submandibular ganglion (submandibular and lingual glands) or in the otic ganglion (parotid gland). Non-myelinated post-ganglionic sympathetic vasoconstrictor fibres and vasodilator fibres supply the blood vessels.

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CHAPTER 20

THE DIGESTIVE SYSTEM (II)

OESOPHAGUS, STOMACH AND INTESTINES

The wall of the tube passing from the mouth to the anus is typically made up of four coats (Fig. 20.1). From the outside these are as follows:

(1) The **serous coat.** The pharynx, the oesophagus and the rectum do not possess this coat, but are attached to the surrounding structures by ordinary fibrous tissue. The rest of the alimentary canal lies less firmly attached in the abdominal cavity, with the surface covered except along its attached border by a serous membrane (the visceral peritoneum) (see p. 78) which forms the suspending mesentery.

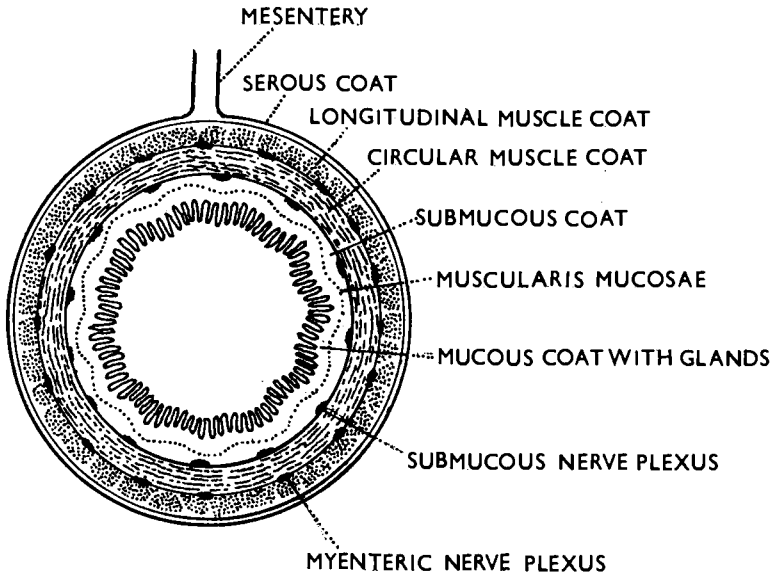

FIG. 20.1.—A diagram of the organization of the gut wall, showing the different layers.

(2) The **muscular coat.** This usually consists of an inner circular and an outer longitudinal layer of smooth muscle: at the sphincters the circular layer is greatly increased. The activity of the muscle coat brings about the onward passage of the alimentary contents, as well as some of the local mixing.

(3) The **submucous coat.** This consists of loose connective tissue containing the large blood vessels and lymphatics.

(4) The **mucous coat.** This is lined by epithelium, and consists of a stroma containing glands and the muscularis mucosae. The *stroma* or *lamina propria* consists of loose connective tissue particularly rich in the usual types of cells together with lymphocytes. The lymphocytes are often aggregated into definite nodules known as solitary follicles: these may extend through into the submucous coat. The *muscularis mucosae* consists of smooth muscle, usually arranged in two layers, an outer longitudinal and an inner circular. It provides strands

running into the mucous coat, and is responsible for most of the local mixing and churning in the alimentary canal.

The *small blood vessels and lymphatics* are found chiefly in the submucous coat.

Dense ganglionated intrinsic *nerve plexuses* (the *enteric plexuses*) are found throughout the canal, situated chiefly between the inner and outer muscle coats (*myenteric plexus* or *plexus of Auerbach*), and in the submucous coat (*submucous plexus* or *plexus of Meissner*). These plexuses are under (extrinsic) parasympathetic control, and supply branches to the various structures, but rhythmic contraction of the gut still occurs even when it is cut off from the extrinsic nerve supply.

PHARYNX

The respiratory and alimentary passages fuse and cross in the pharynx, which is the posterior continuation of the oral cavity. The upper part of the pharynx is the nasal portion, the middle the oral, and the lower the laryngeal. (The nasopharynx and the larynx are described on p. 260.)

The outer **fibrous coat** consists of dense fibro-elastic tissue continuous with the surrounding structures.

The muscle coat lies within the fibrous coat and consists of the striated muscle of the constrictors of the pharynx.

The lining **mucous membrane** is separated from the muscular coat by a dense elastic layer. The stroma consists of fibro-elastic connective tissue containing many lymphocytes and some definite lymphoid nodules (pharyngeal tonsils). Numerous small mucous and mixed glands are present, particularly near the openings of the pharyngo-tympanic (Eustachian) tubes: these may penetrate into the muscle layer. The lining epithelium is of the respiratory type in the upper part, i.e. pseudo-stratified, columnar and ciliated: the cilia tend to disappear with advancing age. A distinct basal lamina separates the epithelium from the connective tissue. The two lower parts of the pharynx are lined by a stratified squamous epithelium continuous with that of the oesophagus.

THE OESOPHAGUS (Figs. 20.2, 20.3)

The walls of the oesophagus are continuous with those of the pharynx and consist of the usual four layers. Except during the passage of a bolus of food, the walls of the oesophagus are closely approximated, so that the lumen appears only as a small, stellate slit in transverse section.

The **external fibrous coat** consists of loose connective tissue continuous with that of the surrounding parts.

The **muscle coat** consists of two layers, of which the outer is mainly longitudinal and the inner mainly circular, although both layers contain many obliquely running bundles of fibres. In the upper part of the oesophagus the muscle is all striated, in the middle part it is mixed, striated and smooth, and in the lower part all the muscle is as a rule smooth. In the region of the cardiac sphincter the circular muscle coat is greatly thickened. Between the two muscle coats is the ganglionated myenteric nerve plexus.

The **submucous coat** consists of dense connective tissue consisting of thick collagenous and elastic networks, and containing the large blood vessels and lymphatics and small aggregations of lymphoid tissue. It is thrown into layers and folds, together with the muscularis mucosae and the mucous coat: these folds are smoothed out when swallowing, this being made possible by the elasticity of the submucous connective tissue. Mucous glands are present in this layer, particularly in the upper part: the ducts pass obliquely downwards to open into the lumen of the oesophagus. A fine ganglionated nerve plexus is found between the submucosa and the circular muscle coat.

The **mucous coat** is separated from the submucosa by the muscularis mucosae. In man the

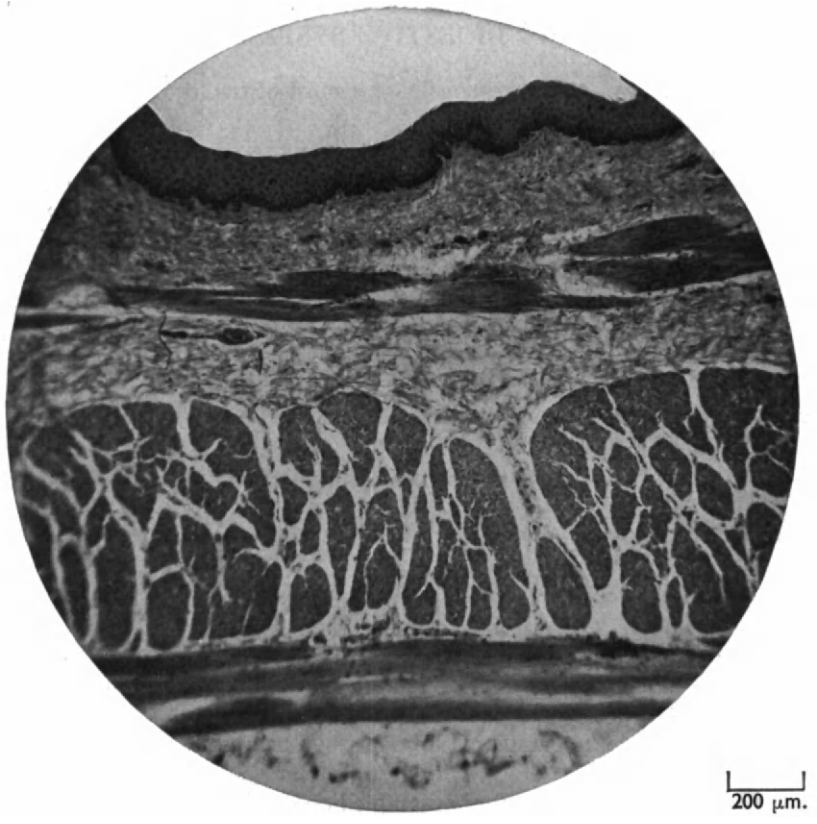

FIG. 20.2.—A longitudinal section of part of the wall of the oesophagus of the cat. There are no glands present in this section; a few of the muscle fibres in the outer longitudinal muscle coat are striated.

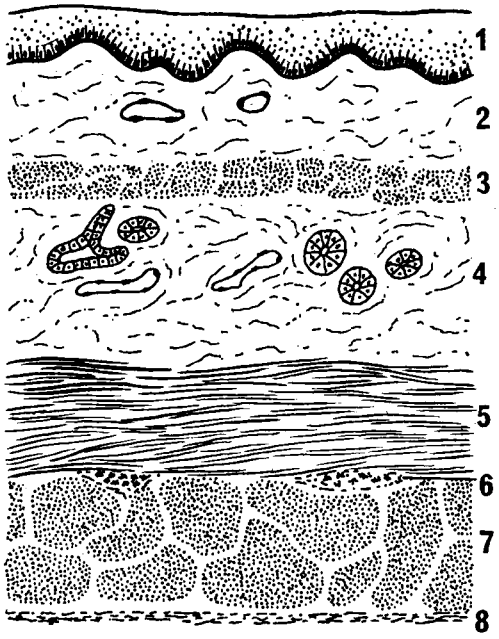

FIG. 20.3.—A diagram of the organization of the oesophageal wall. 1, stratified squamous epithelium; 2, connective tissue of the mucous coat; 3, muscularis mucosae; 4, connective tissue of the submucous coat with mucous glands; 5, circular layer of smooth muscle; 6, myenteric plexus; 7, longitudinal smooth muscle layer; 8, external fibrous coat.

latter is relatively thick (up to 400 μm.), particularly in the lower part: the fibres are arranged longitudinally. The mucosa is lined by a squamous stratified epithelium resting on a layer of connective tissue that projects into it as papillae and contains many scattered lymphocytes. In the upper part, and also near the gastric junction (and occasionally also in other parts) are found branched tubular glands very similar to those of the cardiac part of the stomach. In the region where these glands open the stratified epithelium is replaced by columnar cells.

In the early stages of development the epithelium is ciliated and patches of ciliated epithelium are frequently present at birth. This explains the occasional occurrence of ciliated cells in epithelial tumours of the oesophagus.

THE STOMACH

At the junction of the oesophagus with the stomach (Figs. 20.4, 20.5) the stratified epithelium of the former abruptly changes to the columnar epithelium that lines the rest of the alimentary canal down to the anus. The muscularis mucosae is continuous across the junction, as are also the submucosa and muscle coats. The glands of the gastric coat often extend up under the oesophageal epithelium for a short distance. At the junction the nerve plexuses are particularly well marked and the circular muscle layer is increased in amount to form a rudimentary sphincter. Lymphoid nodules are of frequent occurrence round about the junction.

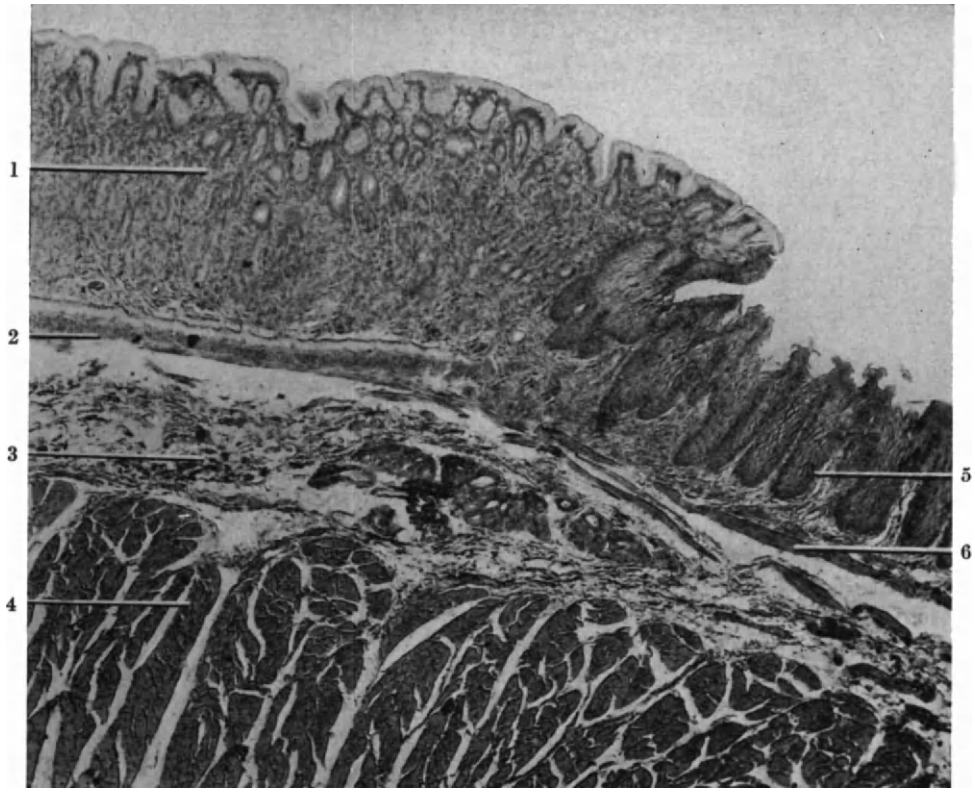

FIG. 20.4.—Longitudinal section through the gastro-oesophageal junction of the cat. 1, mucous coat of stomach with glands; 2, muscularis mucosae; 3, submucosal layer with a group of oesophageal glands; 4, circular smooth muscle layer; 5, stratified epithelium of oesophagus; 6, muscularis mucosae of oesophagus.

The stomach is divided for descriptive purposes into the *cardiac portion* around the oesophageal opening, the *fundus* or main portion and lastly the *pyloric region* which is related to the pylorus or opening into the duodenum. The stomach wall consists of the usual layers.

(1) **Serous Coat.** This is derived from the visceral peritoneum, and consists of a thin layer of loose connective tissue, attached to the external muscle coat and covered with a single layer of mesothelium: it continues into the omentum.

(2) **Muscle Coat.** The muscle coat consists of three rather ill-defined layers, usually described as an outer longitudinal, a middle circular and an inner oblique layer. The outer longitudinal layer is continuous with the longitudinal fibres of the oesophagus: it becomes rather irregular and netlike, and is then gathered into a dense continuous layer that passes over into the longitudinal muscle layer of the intestine. The middle layer is the most regular coat and is greatly thickened, together with the inner coat at the pyloric sphincter.

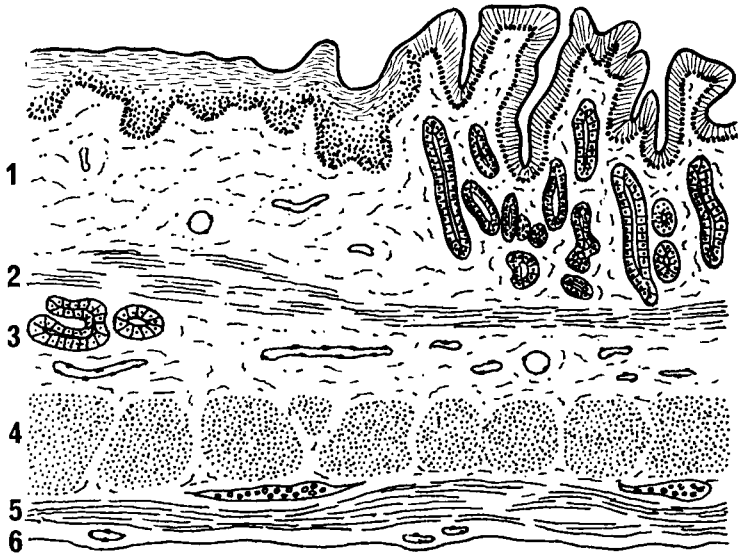

FIG. 20.5.—A diagram of the gastro-oesophageal junction, the stomach is on the right. 1, connective tissue of the mucous coat; 2, muscularis mucosae; 3, oesophageal glands in submucous coat; 4, circular smooth muscle; 5, longitudinal smooth muscle; 6, serous coat.

There is very little change of pressure within the stomach, as the muscular walls respond precisely to the varying volume of the contents: the cavity of the empty stomach is of about the same size as that of the small intestine. Between the muscle groups are found nerve cells and fibres, part of the plexus corresponding to the myenteric plexus.

(3) The **submucous coat** consists of loose connective tissue containing fat cells, lymphocytes, eosinophil leucocytes and mast cells. It contains the blood vessels and lymphatics and a ganglionated nerve plexus

(4) The **mucous coat** (Figs. 20.6, 20.7, 20.8) is separated from the submucosa by a relatively thick muscularis mucosae. This consists of smooth muscle arranged as an inner circular and an outer longitudinal layer: in some parts there is a third outer layer of circular fibres. Strands of muscle extend from the inner layer into the stroma between the glands, their contraction helping to empty the glands.

The whole mucous coat is thrown into ridges (rugae) in the collapsed organ: on distension these disappear and the thickness of the coat diminishes. The lining epithelium is tall, columnar, most of the cells secreting mucin and hence being termed surface mucous cells (Fig. 20.7). These cells are tall, columnar in shape with a vacuolated apical cytoplasm. This is due to the

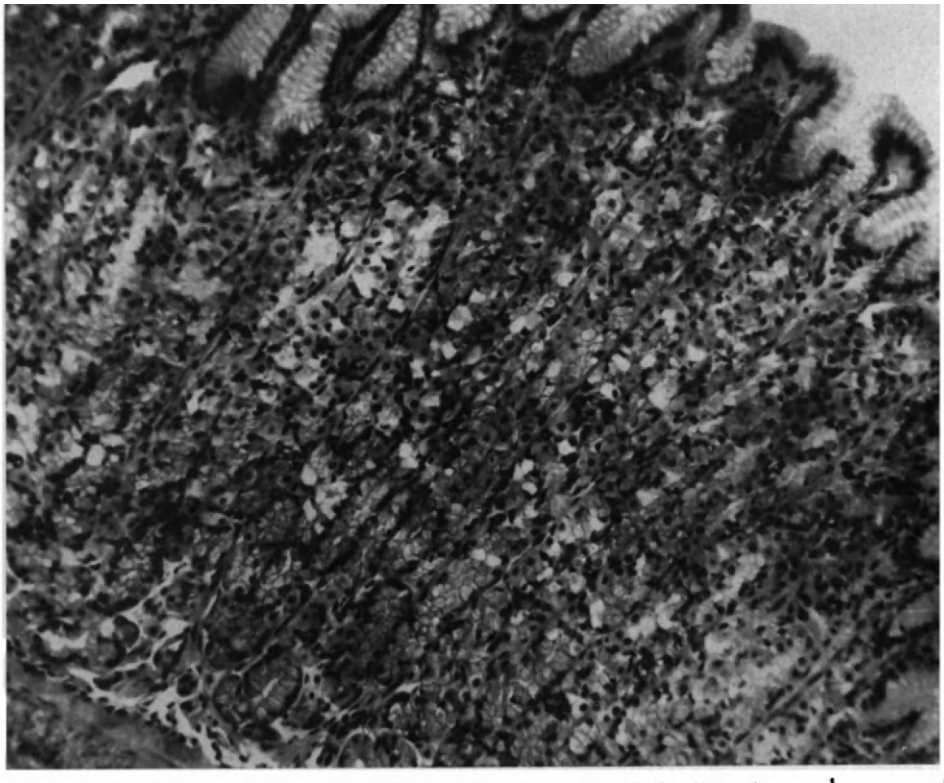

FIG. 20.6.—A micrograph of the mucosal layer of the stomach wall, fundic region. The lumen of the stomach is to the upper right of the micrograph. Note the thick glandular mucosal layer.

75 μm.

FIG. 20.7.—The surface mucous cells which form the gastric lining epithelium. Note the distended palely staining apical cytoplasm of these cells which contain the granules of mucinogen.

20 μm.

solution during the microscopic processing of the droplets of mucinogen which accumulate in this area; if special fixation is used these mucinogen droplets may be characterized by their intense reaction with the PAS technique. The whole surface is studded by the minute openings through which the gastric glands pass out their secretion. The glands occupy most of the thickness of the mucous coat: they are simple branched tubes, perpendicular to the surface, and embedded in the stroma. The small areas between the glands are occupied by fine connective tissue containing fibroblasts, lymphocytes, plasma cells, mast cells, and eosinophil

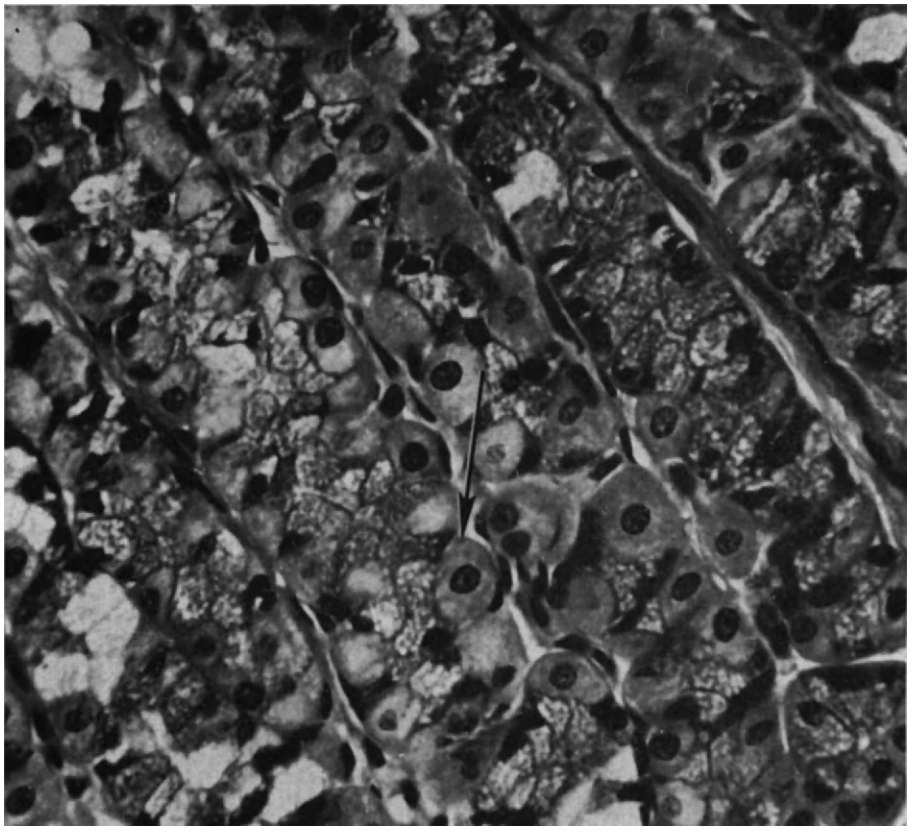

FIG. 20.8.—A high power micrograph of the fundic glands in longitudinal section. The bulk of the cells are chief cells (the secretory granules have not been stained in this preparation); the large spherical cells (one of them arrowed) with central nuclei are oxyntic cells. 20 μm.

leucocytes. The columnar epithelium that lines the wall is continued down into the ducts of the glands: the outer part of the columnar cells usually contains mucin. The gastric glands are of three chief kinds, namely, cardiac, fundic and pyloric.

In addition to its digestive activities, the mucosa of the stomach is important as the site for the synthesis and secretion of *intrinsic factor* which mediates the absorption from the food of vitamin B_{12} (cyanocobalamin), lack of which results in megaloblastic anaemia.

Cardiac Glands

These are found only close to the oesophageal junction. The glands are either simple tubes, like the intestinal crypts, or branching glands lined by cubical cells. The cells seem to be mucus-secreting and resemble those of the pyloric region.

Fundic Glands (Figs. 20.8, 20.9)

These are found throughout the mucosa of the fundus and the body of the stomach. The individual fundic glands are straight running tubes with a narrow lumen and they open, in small groups, into the base of a funnel-shaped depression of the epithelial surface known as a gastric pit. The fundic glands occupy the whole thickness of the mucous layer and the interstitial connective tissue between them is greatly reduced. The glandular cells are mainly of three types, namely, *chief* (*peptic*) cells, *oxyntic* (or *parietal*) cells and mucoid cells. *Argentaffin cells* also occur in small numbers at the base of the glands (see p. 310).

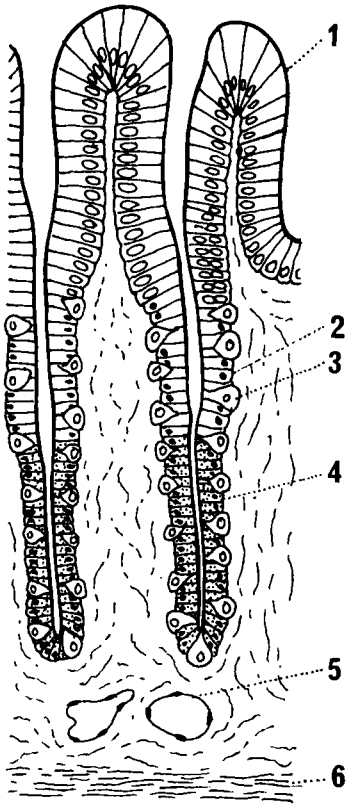

FIG. 20.9.—A diagram of an idealized gastric gland. 1, surface mucous cells; 2, the mucous neck cells; 3, oxyntic cells; 4, chief cells; 5, blood vessel in the lamina propria; 6, muscularis mucosae.

(*a*) **Chief** (peptic or zymogenic) cells. These occur mainly in the lower half of the fundic gland where they are commonly squeezed in between oxyntic cells (Figs. 20.8, 20.9). In shape they are columnar, with basiphilic material rich in cytoplasmic nucleoproteins in the basal part of the cytoplasm and secretion granules in the supranuclear area. The secretion granules are only readily seen after appropriate fixation and staining. They very probably consist in part of pepsinogen, the precursor of pepsin, and in conformity with this they all disappear from the cells when the stomach is stimulated to produce its secretion. The E/M shows the presence of small irregular microvilli on the luminal aspect of these cells, together with large amounts of rough-surfaced endoplasmic reticulum and a prominent Golgi apparatus. Numerous electron-dense secretion granules may often be seen.

(*b*) **Oxyntic** (or parietal) cells (Figs. 20.8, 20.9). These are scattered peripherally along the tubule between the peptic cells and the basal lamina, being more numerous towards the neck of the gland. The epithelium of the gland does not become two-layered but the oxyntic cells are pushed away from the lumen by the chief cells. Typically, an oxyntic cell is large and ovoid

with a central nucleus; the cytoplasm has a marked affinity for acid dyes such as eosin. Recent studies with the E/M have shown that the oxyntic cells are packed with mitochondria (Fig. 20.10). The secretion is passed into the lumen of the gland through canaliculi which represent extensive microvilli-lined invaginations of the plasma membrane of the cells. The oxyntic cells are responsible for the secretion of the acid of the gastric juice. The detailed mechanism by which this occurs is not yet entirely clear; the extensive microvillous surface membrane of the cell undoubtedly plays a major role. Sedar has shown the presence of an extensive mucopolysaccharide layer at the cell surface, which he believes is of special significance in binding

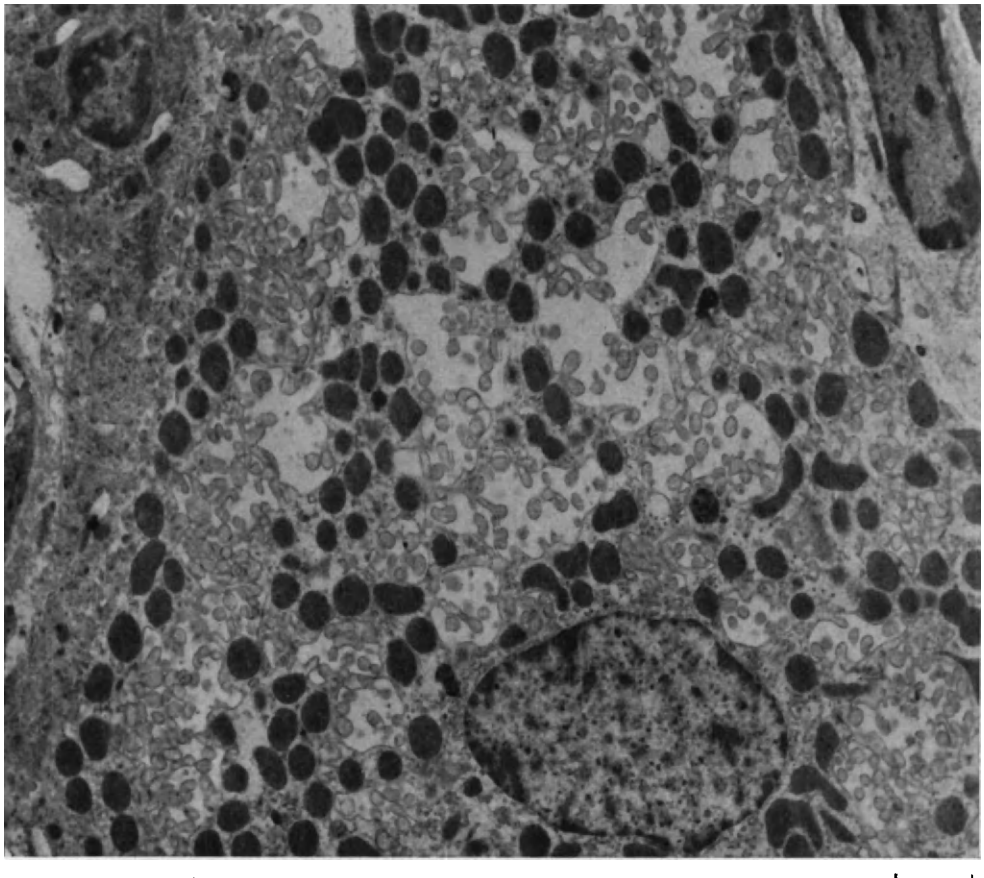

FIG. 20.10.—An E/M of part of cytoplasm of an oxyntic cell from the stomach of a rat. The mitochondria appear as darkly staining bodies. The intracellular canaliculi lined with small microvilli shows well. The nucleus of the cell is at the bottom of the micrograph. 2 μm.

water and so providing a suitable environment for ion transport. The oxyntic cell probably secretes into the canaliculus a fluid which contains a high concentration of sodium chloride. Active transport mechanisms at the cell surface then exchange the sodium ions for hydrogen ions which are derived from breakdown inside the cell of either water or carbonic acid. It may well be very significant that the oxyntic cell is very rich in the enzyme carbonic anhydrase which promotes the formation and breakdown of carbonic acid.

(*c*) **Mucous** cells. (Mucous neck cells.) These cells are found chiefly in the neck of the glands interspersed amongst the oxyntic cells; on passing further down the gland they are largely

replaced by the chief cells. They are larger and clearer than the chief cells, the nucleus is flattened to the base of the cell and the clear granules in the rest of the cell consist of a mucin which is somewhat different chemically from that of the goblet cells of the intestine, for example.

Pyloric Glands

These are found in the pyloric region of the stomach. The pit is long and the secreting part of the gland correspondingly short. The glands are coiled and branched, so that in perpendicular section they are cut through several times. The cells are of one kind only, similar to the mucous cells of the fundic glands. An occasional oxyntic or argentaffin cell may be found.

At the pylorus itself the glands are lengthened and enlarged and may penetrate through the muscularis mucosae, which is here deficient, into the submucous coat.

Blood Vessels and Lymphatics

The stomach differs from the intestine in that it is supplied with arterial blood from both its greater and lesser curvatures. Branches of the large supply vessels pass into the serosa covering the organ, give off branches to the muscle coats of the organ and then form an extensive plexus in the submucosa. Vessels arising from this plexus pass into the mucosa and form a very rich capillary network around the gastric glands. Venous drainage is by a plexus at the level of the muscularis mucosae and by a second plexus in the submucosa; these send main veins out through the muscle layers, again leaving via both curvatures.

The lymph vessels arise as capillary loops or blindly ending sacs at the level of the deep parts of the gastric glands. They pass back and form a mucosal plexus which drains in turn into a larger plexus in the submucosa. From here the lymph passes through the muscle layers into a third or serosal plexus from which the large lymph trunks of the stomach drain back to the large lymph nodes of the greater and lesser curvature.

Cell Regeneration in the Gastric Mucosa

The most recent studies on the gastric mucosa seem to indicate that there is a rapid renewal of the mucous surface and neck cells by migration from a centre of actively-dividing cells in the neck of the gland. It seems, however, that the oxyntic and the chief cells have a long life and are renewed only slowly, probably by differentiation from a stem cell type lying deep in the mucosa.

THE SMALL INTESTINE

The small intestine is concerned with the further digestion of the food and with the absorption of the products of digestion; it is divisible into the duodenum, the jejunum and the ileum. The general structure of the intestinal wall is very similar throughout its length, being characterized by the enormous increase in the surface area brought about by the presence of *villi* or projections of the mucosa. The difference between the intestine and the stomach is seen very markedly at the pyloro-duodenal junction (Figs. 20.11, 20.12) where the typical stomach mucosal arrangement very rapidly assumes the characteristics of that of the duodenum. A thickening of the muscle layers at this point constitutes a sphincter. In the intestine the whole mucosal epithelium and the muscularis mucosae are thrown up into folds, the *plicae circulares* which do not disappear when the wall is stretched (Figs. 20.13, 20.14); the plicae begin about 2–5 cm. from the pylorus.

(1) The **serous coat** (or visceral peritoneum) consists of a layer of mesothelial cells with underlying subserous layers of elastic and collagenous tissue, with the blood vessels exclusively within the deepest layer. At the attachment of the mesentery the serous coat continues on to the surface of the mesentery, the blood vessels, lymphatics and nerves for the supply of the gut passing between the folds.

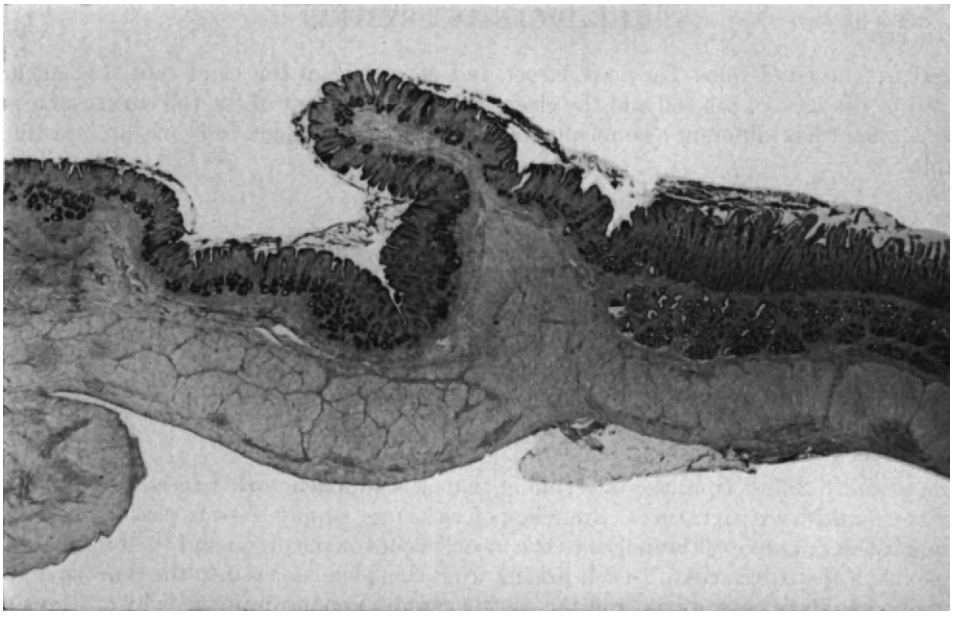

FIG. 20.11.—A photograph of a section through the pyloro-duodenal junction. The pyloric region of the stomach is on the left, the duodenum with the Brunner's glands in the submucosal layer, on the right. Note the pyloric sphincter which appears as a thickening of the muscle layer between the stomach and the duodenum.

500 μm.

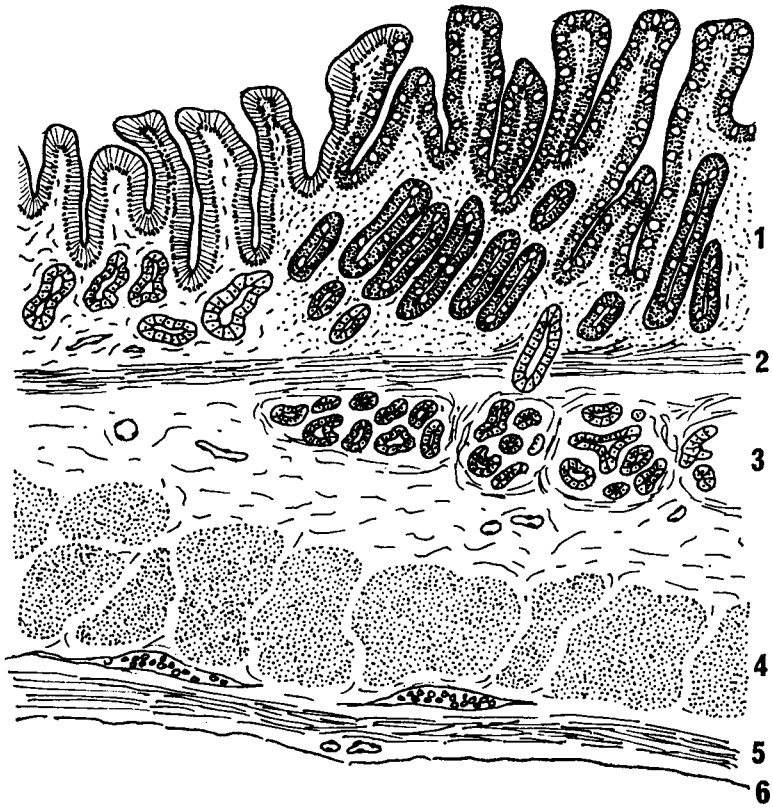

FIG. 20.12.—A diagram of the organization of the pyloro-duodenal junction. The stomach is on the left of the diagram. 1, mucous coat; 2, muscularis mucosae; 3, submucous coat with Brunner's glands in the duodenum; 4, circular muscle layer; 5, longitudinal muscle layer; 6, serous coat.

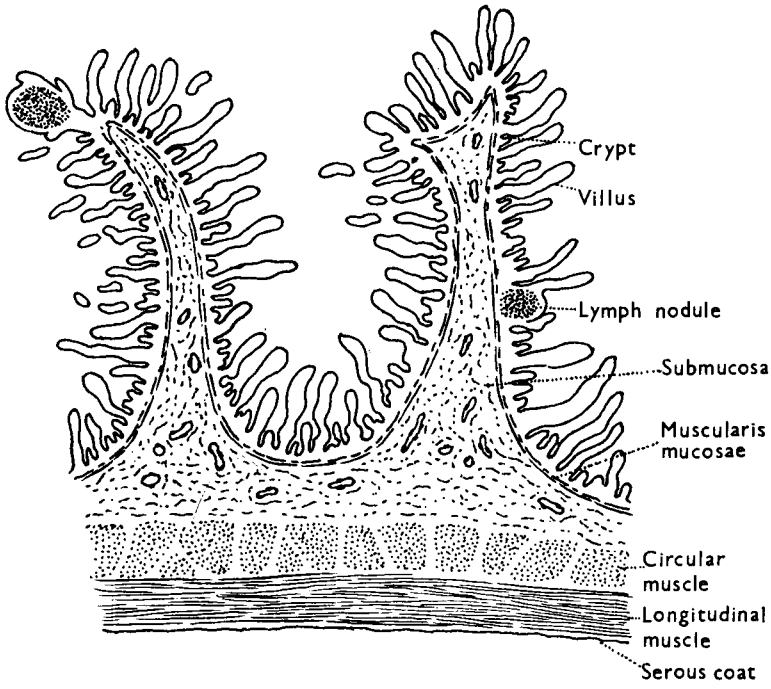

FIG. 20.13.—A diagram of a section through the wall of the small intestine, showing the plicae circulares, one of which has a nodule of lymphatic tissue (stippled) in the mucosa.

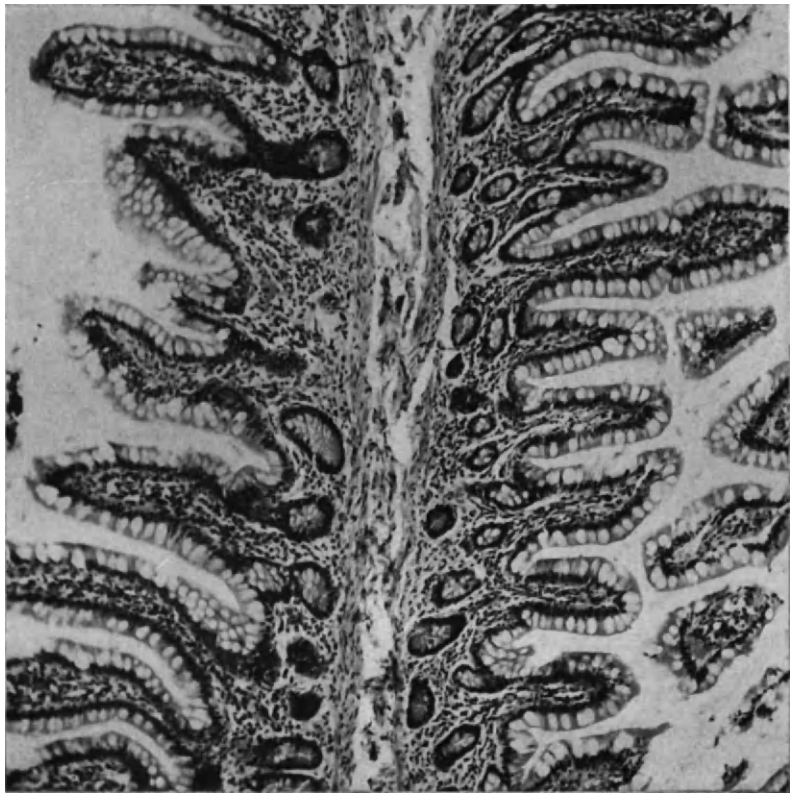

FIG. 20.14.—A micrograph of a plica in the small intestine of the human adult. Note the villi and the numerous goblet cells in their epithelium. 250 μm.

FIG. 20.15.—A spread preparation of the longitudinal muscle coat of the small intestine of a rabbit. This has been treated with gold chloride to show the myenteric nerve plexus. 500 μm.

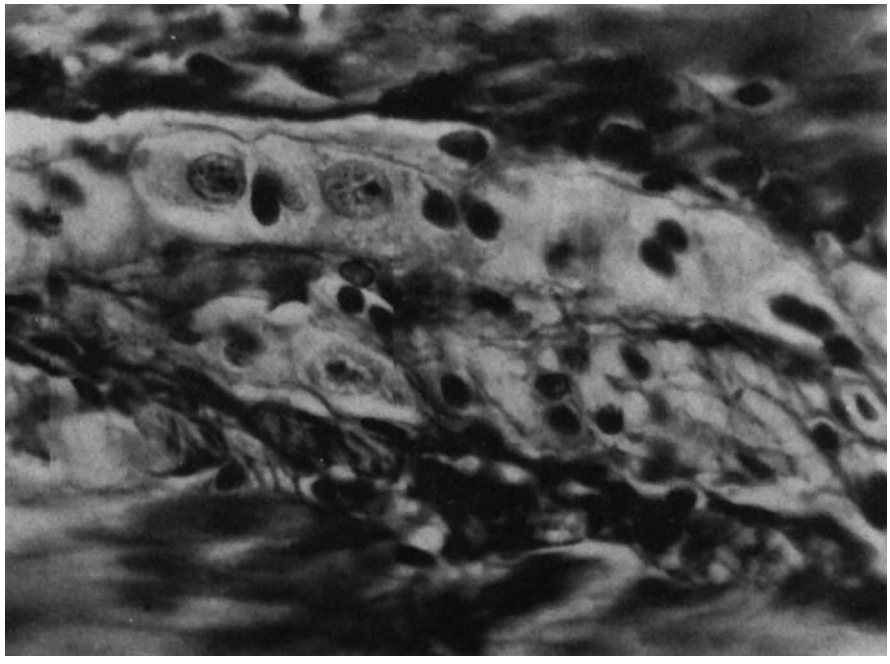

FIG. 20.16.—A high power micrograph of part of a section through the muscle layers of the small intestine wall. This section shows the myenteric ganglion cells in the centre of the field between the muscle layers. 20 μm.

(2) The **muscular coat** consists of well-differentiated external longitudinal and internal circular coats, with blood vessels, lymphatics and the myenteric plexus (Figs. 20.15, 20.16) lying between them.

In reality the muscle layers are composed of bundles of muscle fibres arranged in a helical fashion; the circular muscle being in a spiral with a close pitch whilst the so-called longitudinal fibres have a very coarse pitch. Alternate contractions of these muscles under control of the

myenteric plexus are responsible for the peristaltic movements which transport the gut contents along its length and for the so-called "segmentation" movements which serve to mix the contents thoroughly.

(3) The **submucous coat** consists of connective tissue, with the usual large blood vessels and submucous nerve plexus: adipose tissue is often present. A very dense lymphatic plexus is present in this layer and the efferent vessels drain ultimately into the thoracic duct.

In the *duodenum* only the glands of Brunner occur in this layer (Fig. 20.17). These are racemose glands, with alveoli of clear mucoid and serous cells; their ducts pass up to open at the base of the intestinal glands (crypts of Lieberkühn) (see below). These glands may extend up into the mucous coat and may spread into the pyloric part of the stomach.

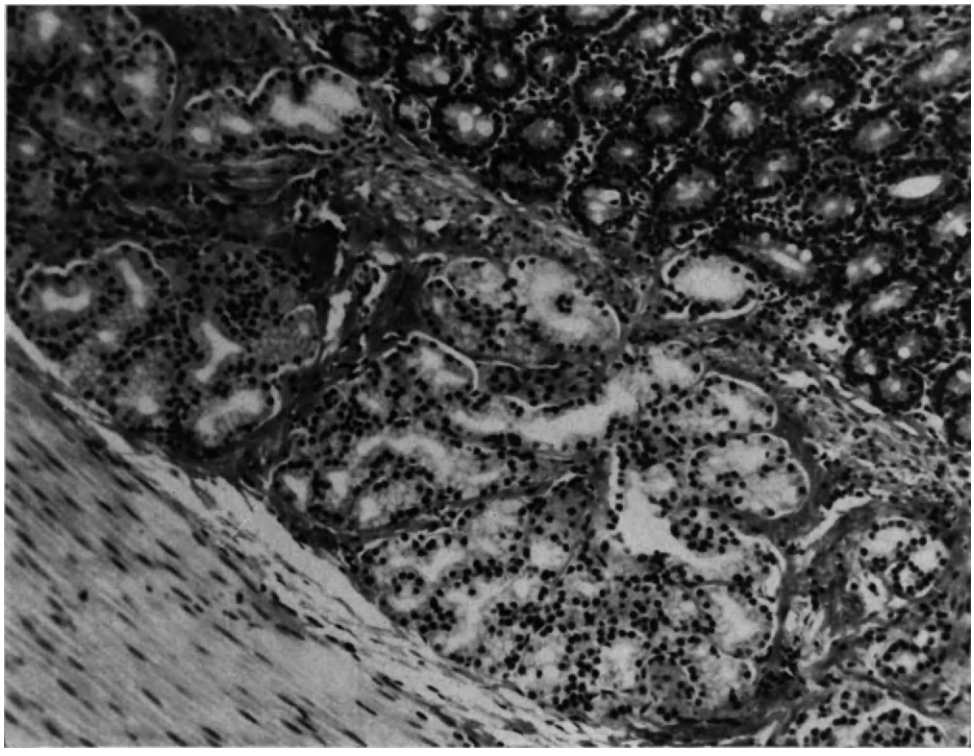

FIG. 20.17.—The submucosal region of the duodenum to show the appearance of Brunner's glands. The mucous coat is at the top right with some of the intestinal crypts in section. The muscularis mucosae is very poorly defined in this section; the circular muscle coat may be seen at the bottom left of the picture. 100 μm.

(4) The **mucous coat** is divided from the submucosa by the muscularis mucosae. This consists of an outer longitudinal and an inner circular layer of smooth muscle, and sends bundles up into the stroma of the villi, where they are attached to the lacteals. The mucous coat is occupied by simple tubular glands, the *intestinal glands* or *crypts of Lieberkühn*, lying closely packed in the *stroma*. The surface of the mucous coat is thrown up into minute projections, the *villi* (Fig. 20.14), which give the surface a velvety appearance: several of the crypts open between two adjacent villi. The surface columnar epithelium covers the villi and the small areas between their bases.

A villus consists of a central core of connective tissue covered by a single layer of columnar epithelium which contains many mucus-secreting cells (the goblet cells) (Fig. 20.14); the

general structure of a villus may be seen in Fig. 20.18. Although the columnar cells usually appear long and thin, their height may vary according to the movements of the intestine; they have a basal nucleus and what appears with the optical microscope as a "striated border". The striated border has been shown by the E/M to consist of microvilli (see Fig. 2.1 and 20.19). The microvilli have been shown to be coated with the filamentous material of the glycocalyx (see p. 14), which in this situation is often referred to as the *enteric surface coat.* It varies from 0·1–0·5 μm. thick and shows the staining reactions of mucoproteins. Recent thinking has tended to consider the enteric surface coat of these cells as the site of activity of many of the digestive enzymes (previously thought to be part of the succus entericus); the enzymes lactase and maltase are almost certainly located in this layer which thus seems

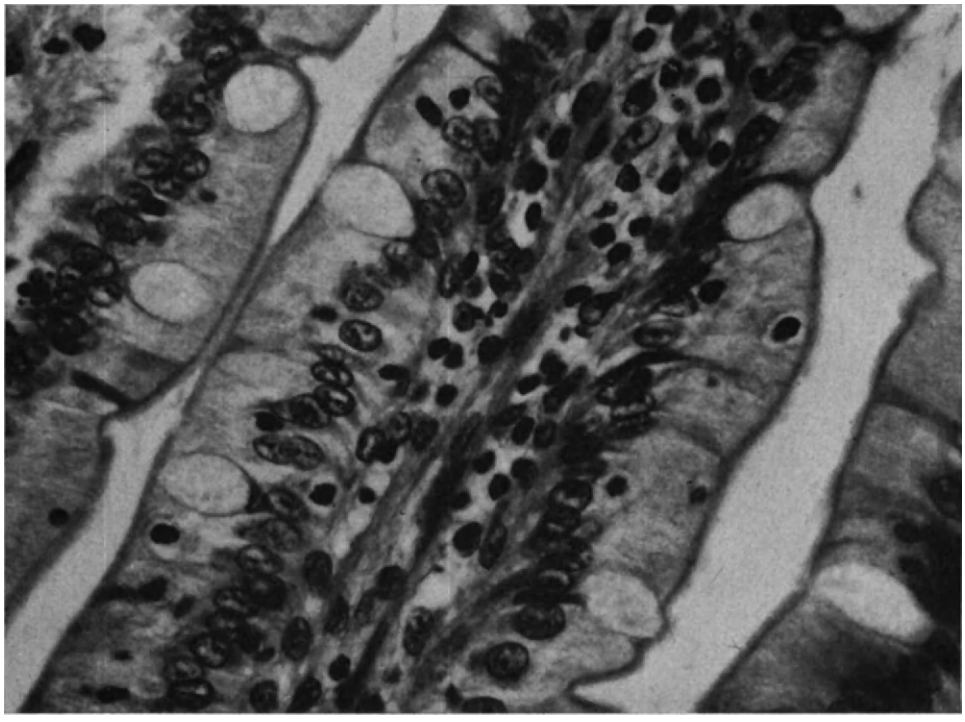

FIG. 20.18.—A micrograph of a part of a sectioned villus in the small intestine. The columnar epithelium with the goblet cells is well shown, together with the central connective tissue core of the villus. 8 μ

to be actively involved in the processes of digestion, as well as increasing the surface area of the cell for purely absorptive purposes and possibly furnishing a specialized micro-environment around the cells. High resolution electron microscopy using material fixed in aldehydes rather than in osmium tetroxide, has shown that each microvillus contains a large number of longitudinal microtubules; these extend into the apical cytoplasm of the cell where they intermingle with a second meshwork of fine filaments orientated parallel to the cell surface and called the *terminal web.*

Each epithelial cell is linked to its neighbour by a well developed *junctional complex* (see p. 40); the epithelial cells are characterized by an extensive network of smooth surfaced endoplasmic reticulum and by numerous mitochondria. Pinocytotic vesicles commonly extend into the apical cytoplasm from between the bases of the microvilli. At one time tiny fat droplets were thought to be taken into the cell by pinocytosis during fat absorption. It is now known,

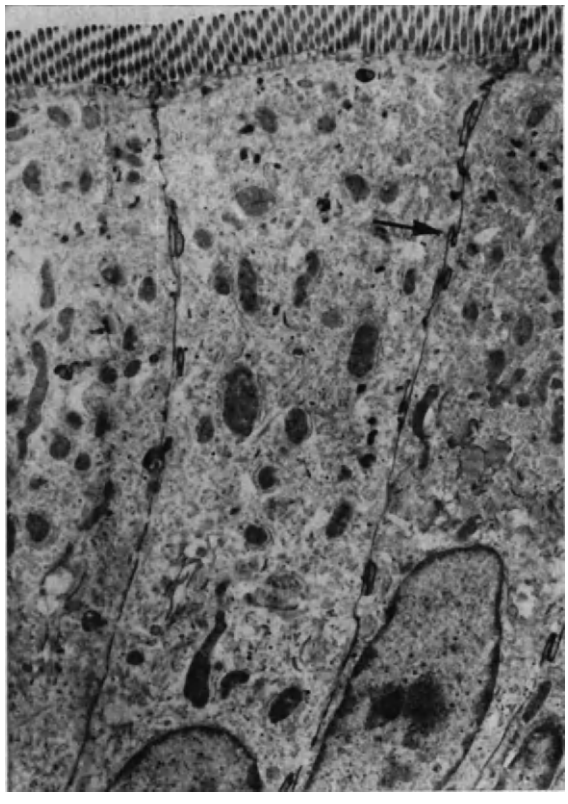

FIG. 20.19A.—A low power E/M of intestinal epithelial cells of the rat. The microvilli on the luminal surface of the cell can be seen at the top of the picture and the cell nuclei at the bottom. The arrow indicates one of the interdigitations of the lateral cell membranes. 1 μm.

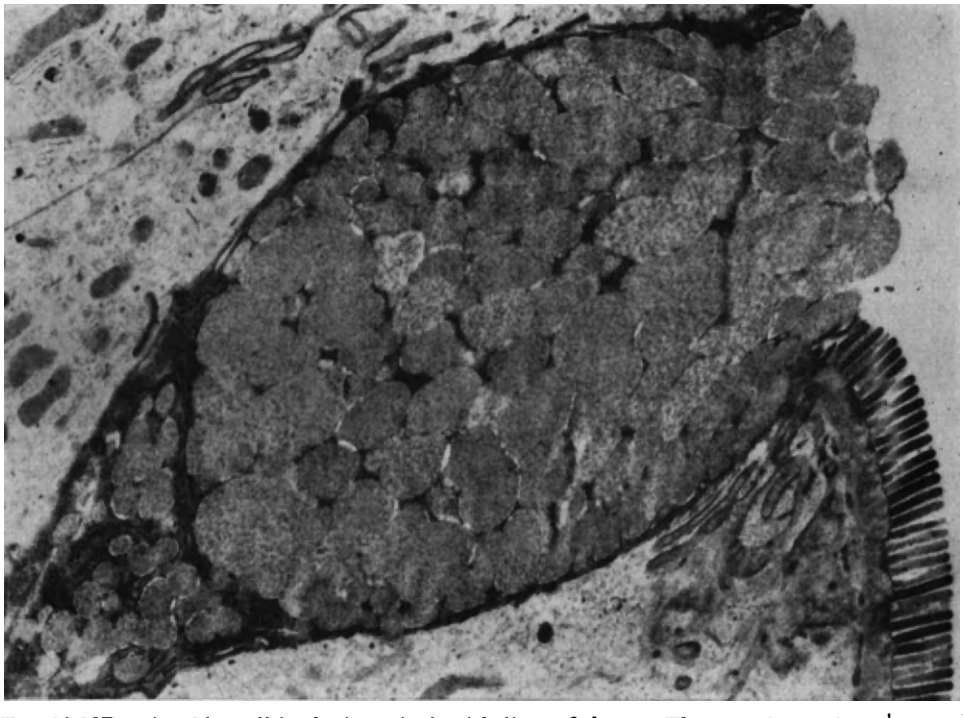

FIG. 20.19B.—A goblet cell in the intestinal epithelium of the rat. The cytoplasm of the cell is very electron dense, unlike the droplets of mucinogen which appear as separate and electronlucent. 1 μm.

however, that the fat is broken up into monoglycerides and fatty acids in the gut lumen and that these substances are actively transported into the cell across the plasma membrane. The monoglycerides appear in the apical vesicles of the smooth surfaced endoplasmic reticulum where they are resynthesized into triglycerides. These are then passed through the cell cytoplasm inside the ER vesicles before they are released into the stroma of the villus and then passed into the central lacteal of the villus. More information on this subject may be found in a paper by Cardell *et al.*

Unicellular gland cells (goblet cells) are scattered irregularly in the epithelium (Figs. 20.18, 20.19B); they show a varying form, according to the state of their secretory cycle. If they are

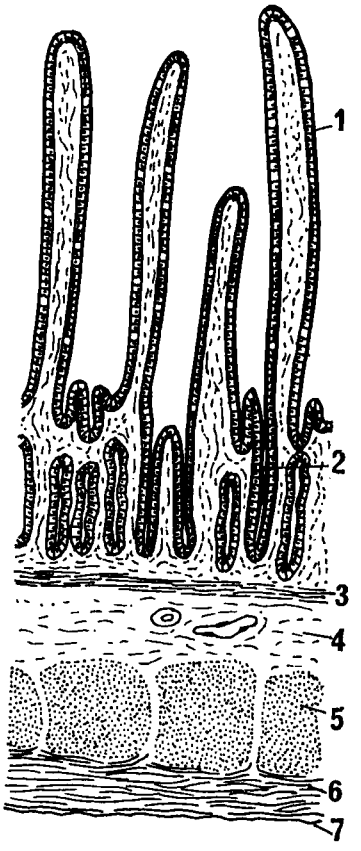

FIG. 20.20.—A diagram of the organization of the wall of the small intestine. 1, columnar epithelium lining a villus; 2, crypt of Lieberkühn; 3, muscularis mucosae; 4, connective tissue of the submucosa; 5, circular muscle layer; 6, longitudinal muscle layer; 7, serous coat.

full of mucinogen droplets the nucleus is often pressed towards the base of the cell. Studies with the E/M shows that they possess a well developed rough surfaced endoplasmic reticulum, with the cisternae often arranged parallel to the lateral walls of the cell. A detailed review of goblet cell fine structure is given by Freeman. The mucus liberated by these gland cells may play a role in the lubrication of the gut contents as they pass down the intestine; certainly the frequency of the goblet cell increases as the contents of the gut become more solid towards the rectum.

In the centre of the intestinal villus is the *lacteal*, a blindly-ending lymphatic passing downwards to the submucous layer: some of the larger villi may contain several lacteals which anastomose one with another. The triglyceride which has been resynthesized in the epithelial cells is transported through the stroma into the lacteal from where it passes through to the

main lymph trunks and then into the cisterna chyli and eventually into the thoracic duct. The presence of large amounts of triglyceride in the lymph after a meal rich in fat gives the lymph a milky appearance. The contraction of the smooth muscle of the villus helps in emptying the lacteals. In a fixed preparation this muscle is contracted and consequently the surface of the villus appears corrugated. Beneath the epithelium is a network of blood capillaries which may be best shown in preparations injected with latex to outline the vessels followed by corrosion away of the tissues. Such a preparation, when viewed with the low power microscope, appears as Fig. 20.21. Here the rich capillary plexus in each villus is apparent together with an indication of the leaf-like shape of each villus.

FIG. 20.21.—A low power micrograph of the injected villi in the human small intestine; the rich capillary network in each leaf-shaped villus is clearly seen. Photograph provided by B. Bracegirdle.

100 μm.

The villi are short and thick in the duodenum, longest in the jejunum, and fewest in the ileum. Recent studies with the scanning E/M have shown that the shape of the villi in the human intestine is very variable, varying from the classical "finger-shape" to leaf-shaped (Fig. 20.21) or occasionally even forming long convoluted ridges. Excellent surface views of villi, obtained with the scanning E/M, will be found in the book by Toner *et al.* listed at the end of this chapter.

Intestinal Glands (*Crypts of Lieberkühn*) (Fig. 20.20). The crypts are lined by a columnar epithelium continuous with the surface epithelium. Large numbers of goblet cells are present among the columnar cells. At the bottom of the gland the cells are lower and show frequent mitoses: these new cells move gradually upwards and differentiate into the goblet cells and columnar cells of the surface, replacing those continually being lost. Also, near the bottom of

the crypt are found the *Paneth cells*, which contain large stainable granules in their cytoplasm (Fig. 20.22). These granules appear to be the precursors of the intestinal enzymes, so that these cells may be included in the general class of zymogenic cells. Occasional *argentaffin cells*, similar to those of the stomach, are also found. These cells are characterized by the presence in a subnuclear position of granules having an affinity for silver. Histochemical results suggest that these granules contain 5 hydroxytryptamine (serotonin) which stimulates the contraction of smooth muscle. The actual role of these cells is not yet clear, but tentatively they may be regarded as endocrine cells located in the gastro-intestinal tract.

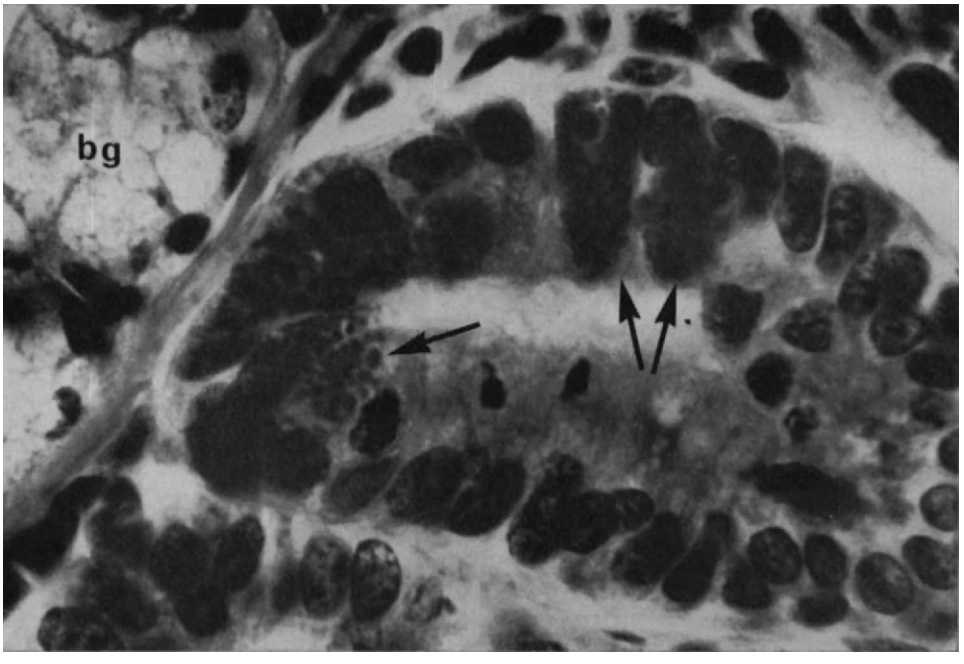

FIG. 20.22.—A high power micrograph of a transverse section of a crypt of Lieberkühn in the duodenum. The arrows indicate three Paneth cells with their serous secretory granules. bg indicates part of Brunner's gland, separated from the crypt by a thin muscularis mucosae. 10 μm.

The *stroma* or *lamina propria* constitutes the core of the villus and fills up all the space between the crypts, and between them and the muscularis mucosae. It consists of fine connective tissue, with a reticular framework like that of a lymph gland, and contains smooth muscle that arises from the muscularis mucosae and runs towards the surface in the core of the villus. A large lacteal is present, as well as a well-developed capillary plexus (Fig. 20.23). In the meshes of the reticulum are found numbers of lymphocytes, plasma cells, mast cells, macrophages and granular leucocytes that are chiefly of the eosinophil variety. Many of the cells migrate through the epithelium, either into the crypts or into the lumen of the gut. Definite nodules of lymphoid tissue occur frequently as the *solitary follicles*. In the *ileum* several of these follicles may be grouped together side by side as *Peyer's patches* or *aggregated lymphoid nodules*: these are visible to the naked eye and bulge on the surface, pushing aside the villi. The lymphoid follicles usually show germinal centres and often spread through the muscularis mucosae into the submucosa (see Fig. 20.24).

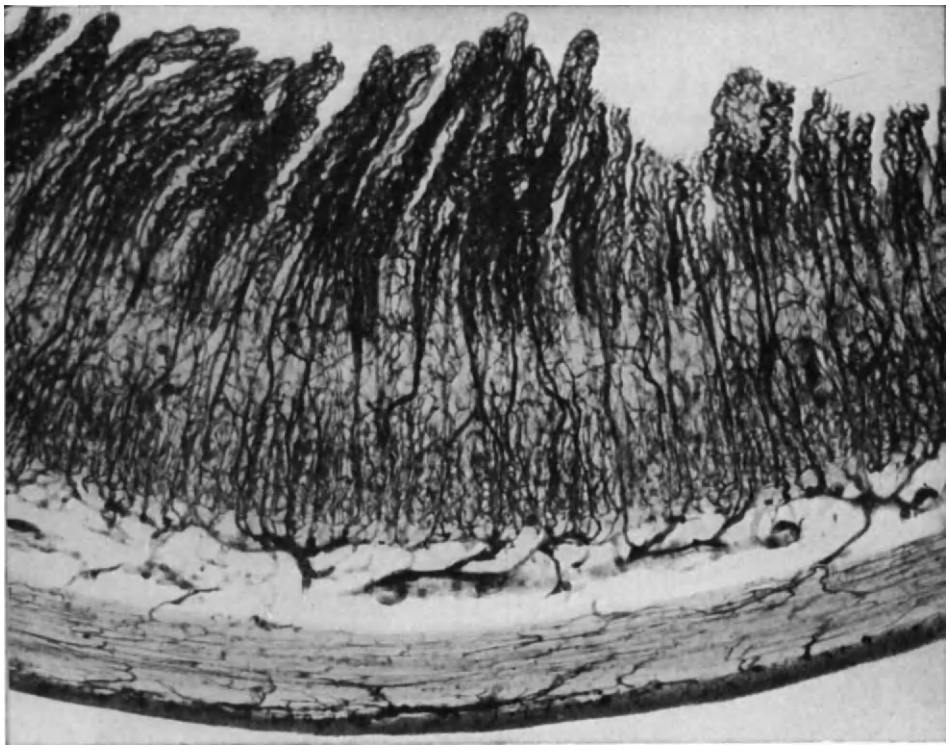

FIG. 20.23.—A transverse section of the small intestine. The blood vessels have been injected and the blood capillary plexus in the villus shows up well. 100 μm.

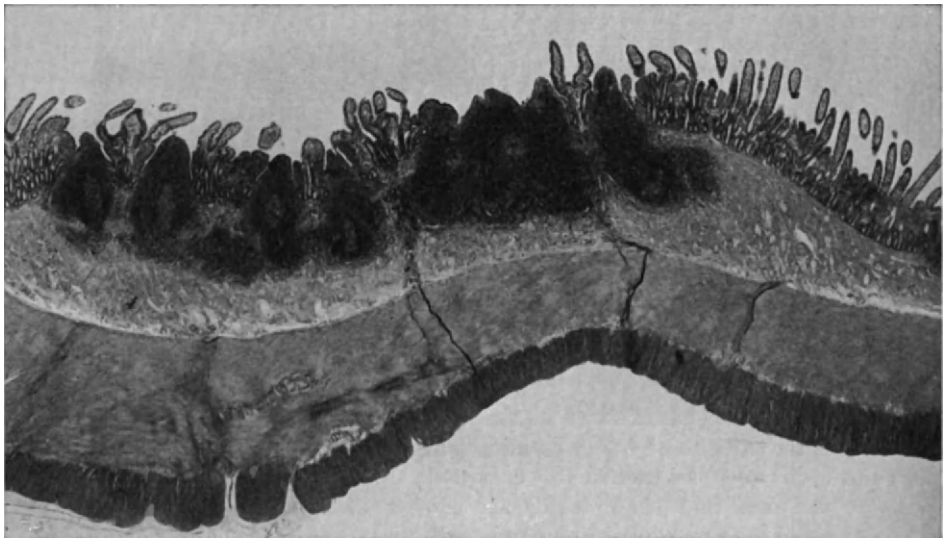

FIG. 20.24.—A transverse section of part of the ileum; the lumen is at the top of the illustration. A large Peyer's patch is visible in the mucosa and submucosa. It appears as a darkly staining mass due to the aggregation of lymphocytes. 200 μm.

THE LARGE INTESTINE, INCLUDING THE COLON, APPENDIX, RECTUM AND ANAL CANAL

Colon (Fig. 20.25)

The usual coats are present in the wall of the large intestine.

(1) The **serous coat** (or visceral peritoneum) consists of loose connective tissue covered by mesothelium. The *appendices epiploicae* of the colon are outgrowths of adipose tissue similar to the omentum.

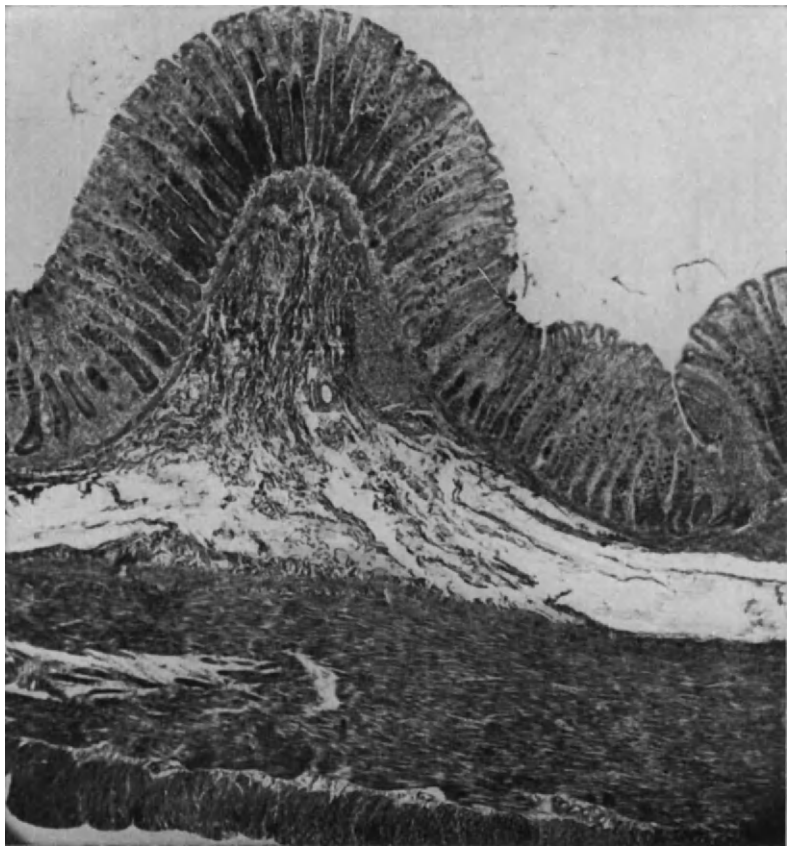

FIG. 20.25.—Transverse section of part of the large intestine of a cat. Note the absence of villi and the numerous deeply staining goblet cells lining the tubular glands of the thick mucosa. The inner circular muscle layer is well marked but the outer longitudinal layer appears very thin in this region. 150 μm.

(2) The **muscle coat** consists of a well-marked inner circular layer, but the outer longitudinal layer is gathered into three thick bands running longitudinally, the *taeniae coli*: this outer coat is complete but in between the taeniae it is extremely thin. The characteristic puckering of the wall is due to the fact that these bands are shorter than the remaining structures. The myenteric nerve plexus is present in the connective tissue outside the circular muscle.

(3) The **submucosa** has the same structure as elsewhere in the gut. The numerous lymphoid solitary follicles lie largely in this layer. The mucous and submucous coats are thrown up into many folds when the longitudinal muscle layer is contracted (see Fig. 20.25).

(4) The **mucous coat** has no villi, but is much thicker than in the small intestine and contains numerous simple tubular glands so closely packed that the intervening stroma is greatly reduced. These glands look like the intestinal glands, and contain large numbers of goblet cells. The epithelium of the colon is chiefly concerned with the absorption of water from the gut contents, so increasing the solidity of the contents. This is reflected in the large numbers of goblet cells in the glands which provide a lubricating coat of mucus to facilitate peristaltic movement of a semi-solid mass of food residues. The deep blind end of the gland is often dilated and possesses the usual proliferating epithelial cells and an occasional argentaffin cell. The free surface between the opening of the glands is covered by columnar epithelium. The muscularis mucosae consists of an inner circular and an outer longitudinal layer.

Vermiform Appendix (Fig. 20.26)

The appendix is a diverticulum of the large intestine and its walls have the same general structure except that the longitudinal muscle coat is evenly distributed round the circumference. It is characterized by a great increase in the lymphoid tissue, the nodules occupying a

FIG. 20.26.—A transverse section through the vermiform appendix. Note the presence of large prominent nodules of lymphoid tissue in the submucosal region.

large part of both mucous and submucous coats: the muscularis mucosae is rather deficient. The numerous eosinophil cells often present in both mucous and submucous connective tissue may indicate some pathological change. The glands are much less closely packed than in the large intestine: they are most numerous in early life and tend to disappear in old age. The lumen of the appendix itself is often obliterated in later life.

Rectum and Anal Canal

The upper part of the rectum (rectum proper) has a structure like that of the large intestine, but the mucous coat is relatively thicker and the glands are longer: it has no mesentery and the serous coat is incomplete. In the lower part of the rectum there are definite longitudinal folds of the mucous coat—the *anal columns* (*rectal columns of Morgagni*): the bases of the columns are connected by transverse mucosal folds, the *anal valves*. At this point, the *ano-rectal junction*, the glands disappear and the epithelium changes to the stratified squamous type (Fig. 20.27).

The muscle coats are continuous and well developed, and just above the anus the circular coat is thickened into the internal sphincter: the external anal sphincter consists of striated muscle.

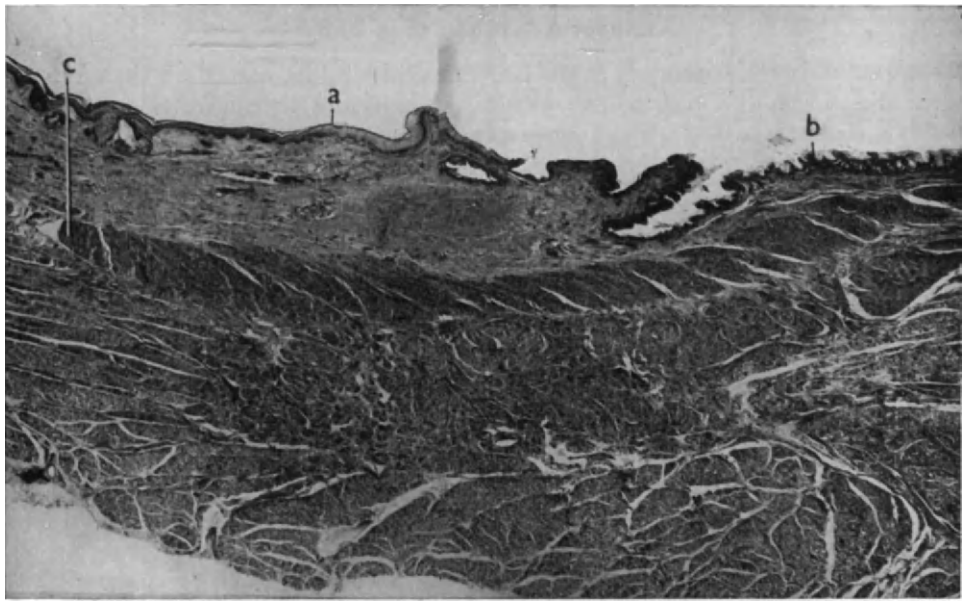

FIG. 20.27.—A micrograph of a longitudinal section through the ano-rectal junction (the rectum is on the right). Note the stratified squamous keratinized epithelium continuous with that of the skin on the left at a, and the tubular mucous glands of the rectum at b. The termination of the smooth muscle of the gut is indicated at c. 150 μm.

In the neighbourhood of the anus there are sebaceous glands and numerous racemose sweat glands, the large apocrine circum-anal glands. The corium contains many large veins: when these are unusually dilated they may appear as haemorrhoids or piles.

Cell Regeneration in the Small Intestine

Autoradiographic studies with tritium-labelled thymidine have shown that there is a rapid replacement of the absorptive epithelial cells of the small intestine. The new cells arise in the base of the crypts, where numerous mitotic figures may be seen, and migrate up the glands and from the base to the apex of the villi. They are desquamated from the apex of the villus into the intestinal lumen after a life of about two days.

The Rôle of Mucus in the Alimentary Canal

Mucus secreting cells are common throughout the alimentary tract; the mucoprotein which they secrete is characterized not so much by its chemistry as by the fact that it is always very viscous and so is well suited to forming coatings on surfaces. In the mouth, isolated mucous glands and the mucous portions of the submandibular and sublingual glands produce

a secretion which is mixed with the food during mastication. This undoubtedly helps as a lubricant in the process of swallowing and in the passage of the bolus of food down the oesophagus.

In the stomach the whole of the surface epithelium is modified to secrete mucus; here it has been suggested that the primary function of the mucus is to form a thin tenacious coating over the epithelium and so prevent damage to the cells by the high concentration of acid produced by the gastric glands. In the region of the pylorus and the duodenum it seems likely that the high content of bicarbonate ions which accompany mucus secretion play a significant rôle in the neutralization of the gastric juice.

Measurements show that the number of mucus-secreting goblet cells in the gut increase in number according to the distance from the pylorus.

This has led some workers to suggest that the mucus here is more important as a lubricant allowing easy peristalsis when the gut contents are becoming semi-solid as they approach the rectum. Observations that the goblet cells discharge when in contact with an irritant (either chemical or mechanical) suggest, however, that this is not the full story. Florey summed up the possible rôles of mucus in the gut by commenting that it was essential in order to dilute an irritant, to push irritants away from the microvillous borders of the epithelial cells, to help "wrap up" solid contaminants and undigested solid material and to help in the process of the evacuation of undigested material.

Post-mortem Change in the Alimentary Canal

During life the mucous layer of the wall of the alimentary canal can protect it from the action of the digestive juices, of putrefactive micro-organisms and of chemical products of digestion within its lumen. After death this protection ceases and the wall undergoes rapid autolysis. Desquamation of the lining epithelium usually begins within an hour of death, and the changes spread rapidly outwards until all coats of the wall are involved. (For this reason it is more convenient to study the minute structure of the alimentary canal in material taken from animals rather than from the human.)

Lymphoid Tissue in the Alimentary Canal

The various components of the lymphoid nodules react in the same way as lymphoid tissues elsewhere in the body. A particularly interesting reaction is the lesion of the Peyer's patches in typhoid fever due to the multiplication of the typhoid bacilli in the lymphoid tissue; necrosis and perforation or haemorrhage may sometimes follow. Another feature of the Peyer's patches is the presence of large numbers of parallel blood vessels running into them from the submucosal plexus: this accounts for the severity of the haemorrhage that may occur from even superficial ulceration of such a patch.

The increase in the amount of lymphoid tissue as one passes from the stomach to the anus reflects the bacterial content of the gut. The strongly acid stomach contents render this region virtually free from bacteria, but as the lower part of the ileum is reached more and more bacteria appear. This reaches a maximum in the lower colon and rectum, both of which are very rich in lymphoid tissue.

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CHAPTER 21

THE DIGESTIVE SYSTEM (III)

THE LIVER AND THE PANCREAS

The Liver*

In man, the liver arises as a diverticulum of the gut endoderm just anterior to the anterior intestinal porta. Cells proliferating from the diverticulum pass into the mesenchyme of the septum transversum and occupy the meshes of an already existing network of capillaries related to vitelline and umbilical systems of vessels. Simultaneously there is an invasion of the same spaces by cells derived from the visceral mesothelium. Both kinds of cells together form the hepatic parenchyma.

The liver is surrounded beneath its serous covering by a connective tissue capsule, formerly known as *Glisson's capsule*: at the hilum this capsule penetrates into the organ, giving off septa. Trabeculae from these septa and from the capsule pass into the lobes, dividing them into *lobules* (Fig. 21.1): in man these trabeculae are very incomplete. The morphologically recog-

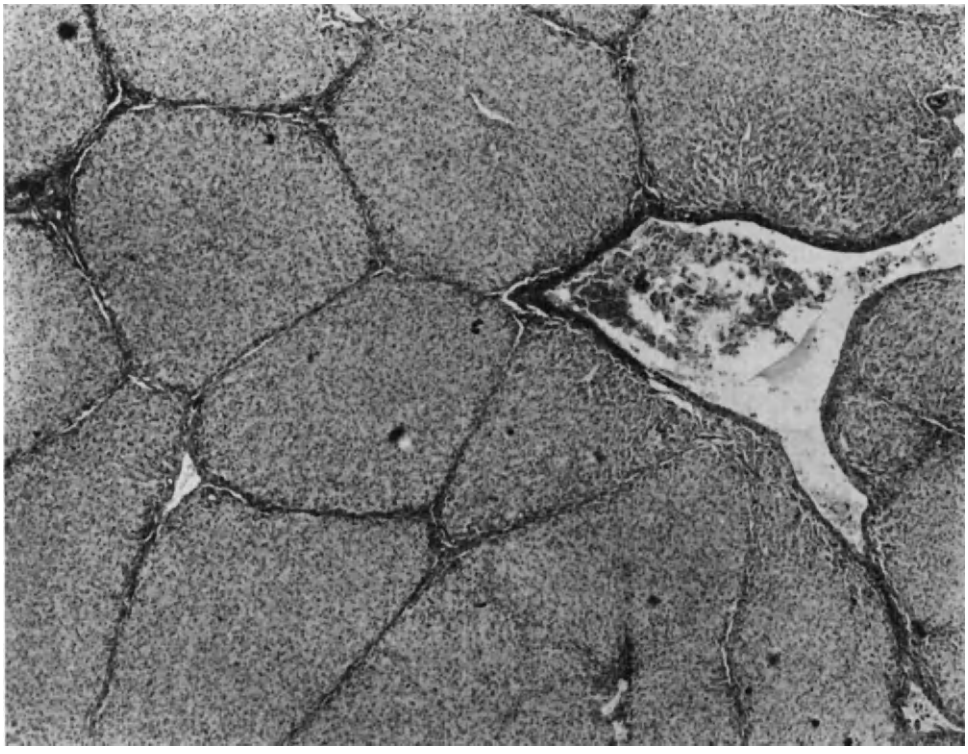

FIG. 21.1. A low power micrograph of the liver of the pig, in which the trabeculae separate the parenchyma into very marked lobules. A large branch of the portal vein with two tributaries is visible on the right of the picture. 250 μm.

* The account of the liver given here is based upon the investigations of Rappaport and of H. Elias and his collaborators.

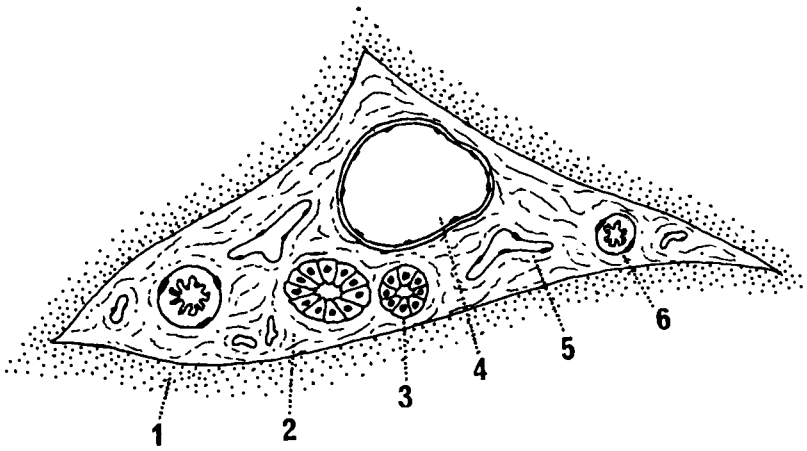

FIG. 21.2. A diagram of the structures normally found in the portal canal (the connective tissue between the liver lobules). 1, liver lobule; 2, connective tissue; 3, interlobular branches of bile duct; 4, interlobular vein (branch of portal vein); 5, lymphatic; 6, branch of hepatic artery.

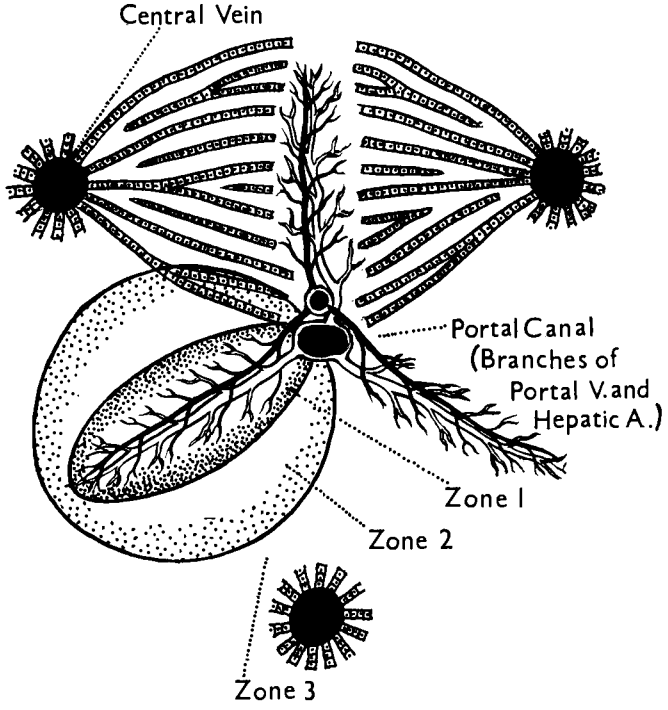

FIG. 21.3. A diagram of Rappaport's concept of the hepatic acinus. This is the tissue of adjacent morphological lobules that is supplied by a terminal branch of the portal vein and hepatic artery. At the bottom left the three physiological zones of differing oxygen concentration are indicated, zone 1 being the zone of the highest concentration.

nizable lobule was formerly regarded as the unit of the liver structure. The lobules are polyhedral, usually showing five to seven sides in a cross-section. Running through the middle of each lobule is the central vein (*intralobular vein*), which drains away via a radial network of sinusoids into a sub-lobular tributary of the *hepatic vein* (see also p. 321). At the periphery of the lobule, in the connective tissue, are found the *interlobular vein* (which is a tributary of the

hepatic portal vein), branches of the *hepatic artery*, the *interlobular bile ducts* and *lymphatic vessels*: the whole is termed a *portal canal* (see Fig. 21.2).

The studies of Rappaport have suggested that the liver may best be regarded as being composed of functional units which cannot be equated with the morphological lobules. Rappaport introduced the concept (based upon the earlier work of Mall) of the *liver acinus* which may be defined as that mass of liver tissue which is supplied by a terminal branch of the portal vein and hepatic artery and drained by a terminal branch of the bile duct. This is illustrated in Fig. 21.3. There are, in Rappaport's system, no anatomical boundaries between

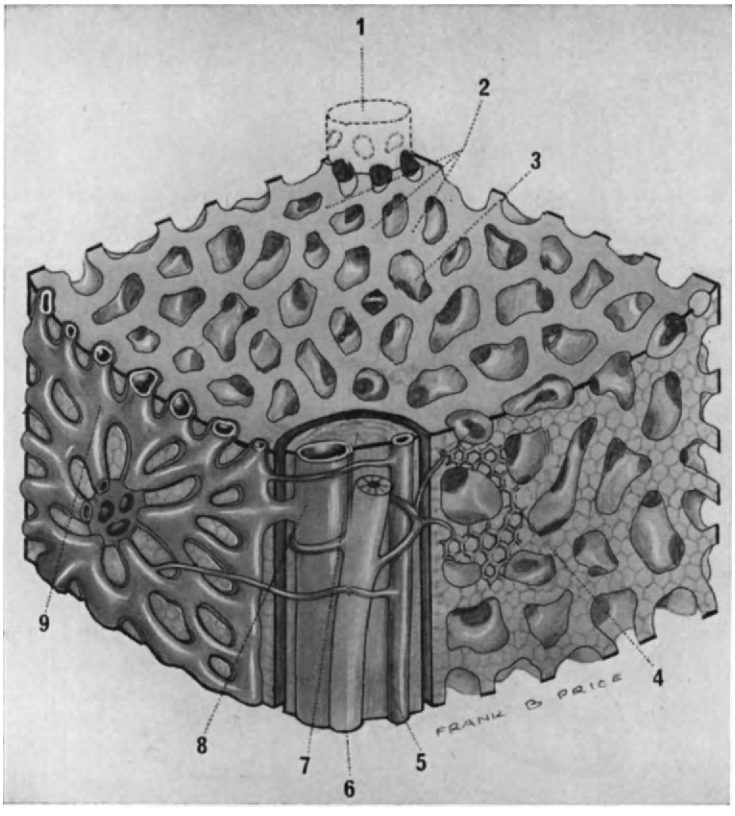

FIG. 21.4. A diagram of a block of liver tissue to show the muralium or wall-like structure. 1, intralobular vein; 2, muralium of liver parenchyma cells; 3, lacunae; 4, terminal branches of bile ducts; 5, branch of hepatic artery; 6, interlobular branch of bile duct; 7, connective tissue in the portal canal; 8, interlobular vein (tributary of the portal vein); 9, sinusoids occupying the lacunae between the muralium and draining into the interlobular vein.

one acinus and the next and an acinus would normally comprise the adjacent portions of two of the morphological lobules. There is a physiological zonation in the acinus, indicated in the figure, due to the variations in the oxygen content of the blood reaching the various parts and in the differing content of nutriments in the blood reaching the zones.

Hepatic Cells

The liver as a whole consists of a continuous mass of cells traversed by a complex system of anastomosing tunnels or *lacunae*. The lacunae are occupied by a network of blood-containing sinusoids, themselves in communication with the hepatic veins, portal veins and hepatic arteries. The liver cells separating adjacent lacunae (see Fig. 21.4) form branching and anasto-

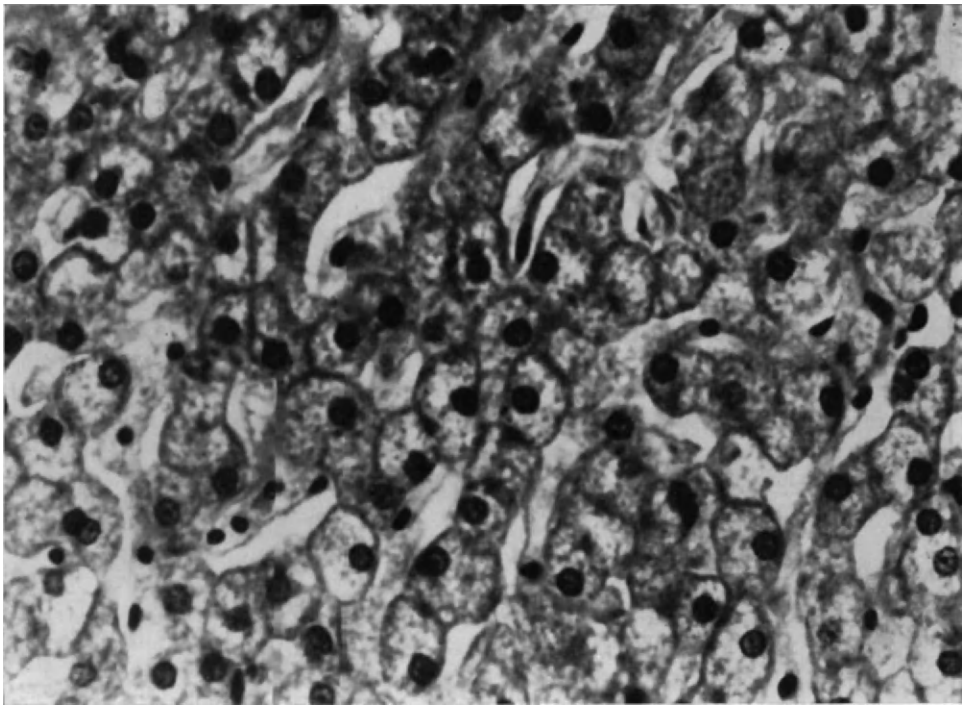

FIG. 21.5. A micrograph of liver parenchymal cells. The sinusoids between the cells are visible. The "empty" appearance of the cells is due to the loss of their glycogen during the processing. 15 μm.

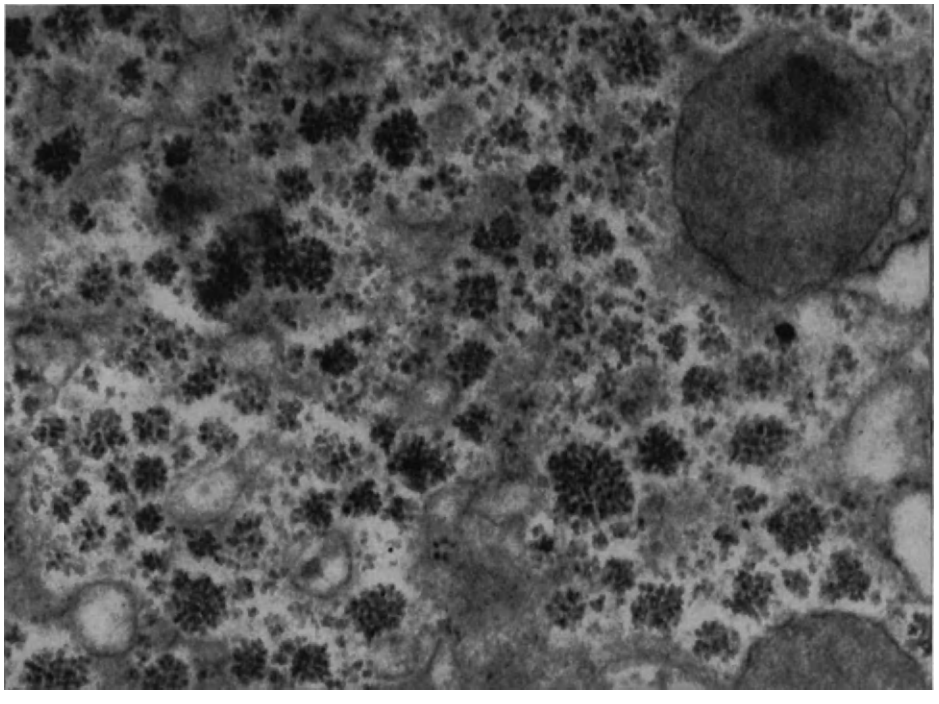

FIG. 21.6. An E/M of liver cell cytoplasm, showing the darkly staining rosettes which are the glycogen deposits. Vesicles of the smooth surfaced ER may be seen at the lower left and a microbody (similar to a lysosome) is at the upper right of the micrograph. 0·25 μm.

mosing plates, rather like walls, and the term "muralium" has been suggested as best describing the manner in which the liver cells are arranged in the hepatic parenchyma.

The liver cells appear to be all of the same type; they are polyhedral in form, sometimes binucleate, and in stained preparations the cytoplasm often appears coarsely granular or "empty" (Fig. 21.5). The nucleus is large, round and vesicular. Glycogen (Fig. 21.6), lipid and iron are to be found in varying amounts, depending upon the functional state of the cells. Where the cells of the liver parenchyma are in contact with the portal canals and the external capsule a continuous sheet of cells exists which has been called the limiting plate. Proximal to the central veins of the lobules the limiting plate is interrupted by sinusoids (see below) discharging directly into the central vein.

Liver cells normally possess not only a well developed rough-surfaced endoplasmic reticulum, which accounts for the extensive cytoplasmic basiphilia due to RNA, but also considerable amounts of smooth ER as well. The mitochondria are numerous and vary in shape from ovoid to rod-like and microbodies are present (see Fig. 21.6). Microvilli occur on the surfaces of liver cells adjacent to the sinusoids, thereby increasing the surface area both for secretion and absorption (Fig. 21.10).

Bile Canaliculi (Fig. 21.7)

Associated with the liver muralium are the *bile canaliculi*. These are very small when seen in section with the optical microscope, often being from 0·5–1·0 μm. in diameter. The E/M has

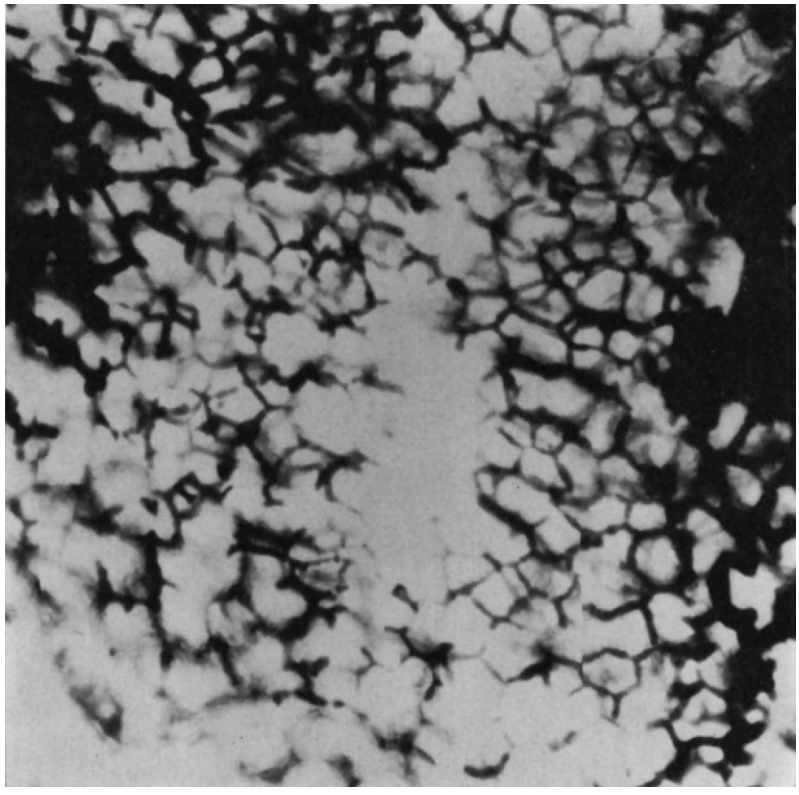

FIG. 21.7. Bile canaliculi between liver parenchymal cells. They have been visualized by the retrograde injection of a dye up the bile duct. Note their extensive anastomoses; the liver cells are not shown by this technique. 10 μm.

shown that here they lack a lining epithelium, the membranes of two adjacent parenchyma cells separating to form the space of the canaliculus (Fig. 21.8). Above and below the canal so formed the cell membranes are sealed together by tight junctions which resemble the zonulae occludentes of the junctional complex (see p. 40). These doubtless prevent the bile from seeping away through the intercellular matrix. The surfaces of the liver cells which form the bile canaliculi are folded into small microvilli which project into the lumen of the canal (Fig. 21.8). Alkaline phosphatase can readily be demonstrated in association with these structures in tissue sections. The canaliculi, draining outwards, are connected to the bile ducts running in the portal canals by very tiny ductules (see Fig. 21.4). The interlobular bile ducts of the portal canals are lined by a columnar epithelium resting on a basal lamina, and in the larger ducts there is connective tissue and smooth muscle in the wall. In addition, there are intralobular bile ductules lined by a very flattened epithelium. These are very small and not easily seen.

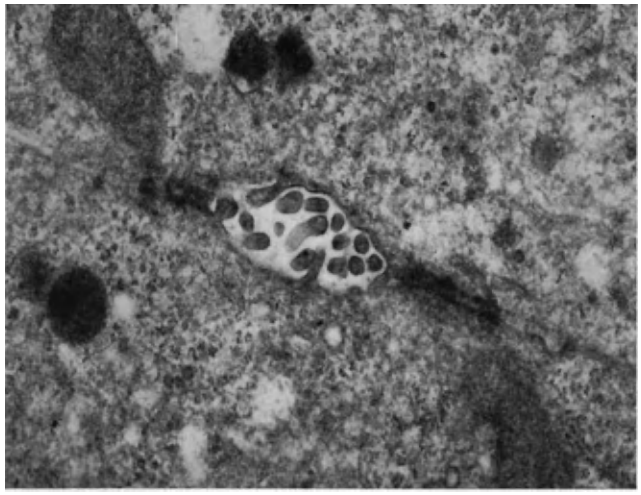

FIG. 21.8. An E/M of the plasma membrane between two adjacent liver cells. The unit membranes of the cells diverge to form the bile canaliculus (in the centre of the micrograph). Microvilli project into its lumen and the membranes are sealed together on either side by darkly-staining zonulae occludentes. 0·25 μm.

It is clear that the liver is an exocrine gland with a very individual type of morphology. The aggregations of ducts (or ducts and alveoli) which characterize all but the simplest exocrine glands are here represented by a muralium of secretory cells. With regard to the duct system, it would appear that only the interlobular bile ducts of the portal canals and the larger ducts derived from them show a general histological similarity to the duct system of a typical tubuloalveolar gland such as the pancreas. Both the intralobular ductules and the bile canaliculi themselves lack exact counterparts among the latter kind of gland.

Blood Vessels

The hepatic artery supplies the large bile ducts and the gall-bladder, and within the liver itself the connective tissue and the walls of the big veins and the bile ducts, but only to a small extent the hepatic cells. The portal vein is the chief afferent vessel, and supplies most of the blood to the liver substance. At the porta of the liver the capsule encloses the entering portal vein and hepatic artery and the emerging bile ducts, and also lymphatic vessels and nerves: this connective tissue continues into the liver providing a sheath for all these structures in their ramifications between the lobules, the whole being known as a *portal canal*

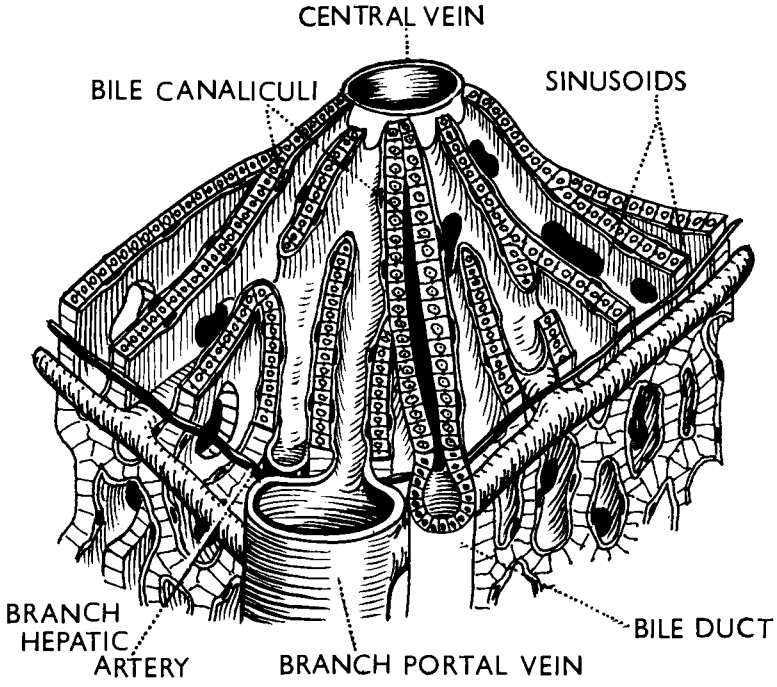

FIG. 21.9. A diagram of the vascular pattern in a liver lobule. The interlobular branches of the portal vein open into the sinusoids between the liver cells. A bile duct is also shown in the portal canal, receiving a bile canaliculus from between two rows of liver cells. The sinusoids open into the central (intralobular) vein which drains in turn into the hepatic veins.

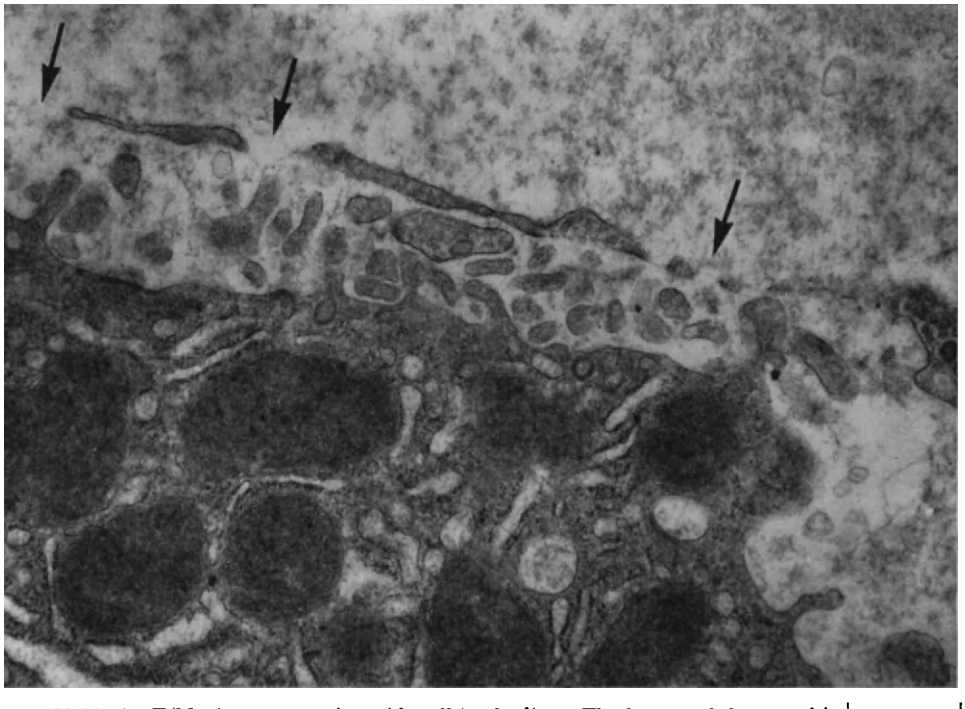

FIG. 21.10. An E/M of part of a sinusoid wall in the liver. The lumen of the vessel is at the top of the picture. Note how the sinusoidal endothelium rests on the tips of parenchymal microvilli, so forming the peri-sinusoidal space or "space of Disse". Three of the many discontinuities in the endothelium are indicated by the arrows. 1 μm.

(Fig. 21.2). The two afferent vessels (hepatic artery and portal vein) both give rise to interlobular branches; those from the portal vein form the inlet venules which open into the sinusoids between the muralium of the hepatic cells (Fig. 21.9).

The sinusoids are lined by a discontinuous sheet of cells (Fig. 21.10) the nature of which was in doubt for many years. Current opinion based on studies with the E/M and on experimental approaches suggests that there are present both typical endothelial cells, with small nuclei, and the cells classically described as *stellate cells of Kupffer* (Fig. 21.11). These latter cells are to be regarded as fixed macrophages. They frequently contain pigment granules and

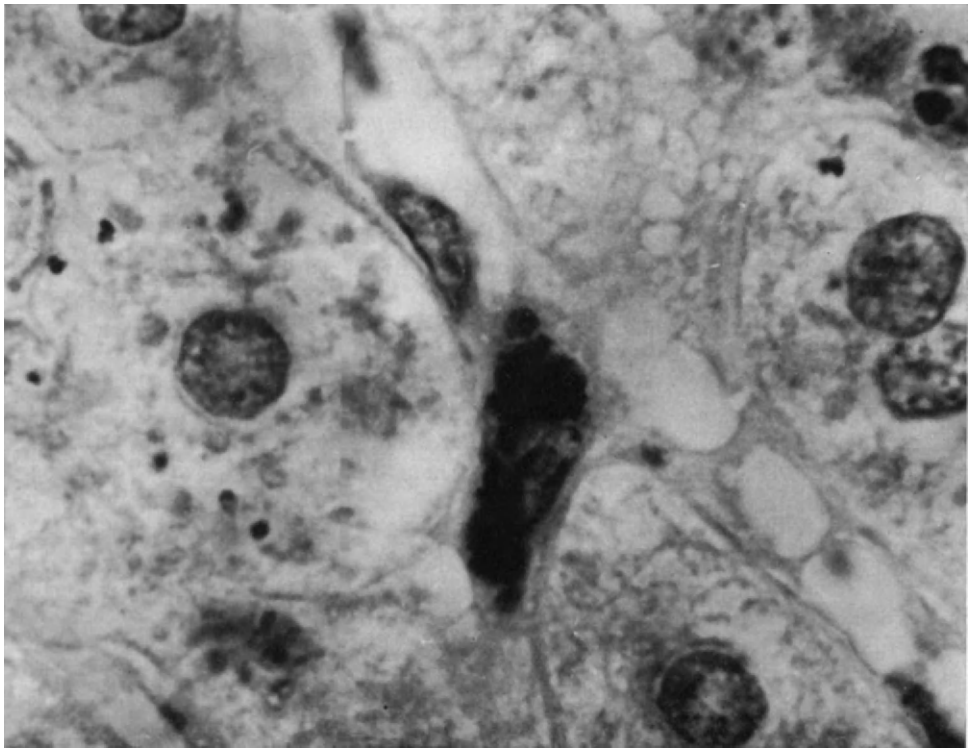

FIG. 21.11. A high power micrograph of liver parenchyma from an animal which had received an injection of trypan blue. The single large Kupffer cell in the centre of the picture has accumulated a large amount of the dye in its cytoplasm. A nucleus of an endothelial cell is visible just above the Kupffer cell. Note also that isolated dye particles have accumulated in the lysosomes of the liver cells.

erythrocyte debris and they are capable of engulfing particulate matter injected into the blood stream. The E/M has shown that there are large breaks in the endothelial cell lining of the liver sinusoids (Fig. 21.10) although a recent study by Wisse, using a perfusion technique of fixation, suggests that a more typical appearance would be of a thin plate of endothelial cell cytoplasm perforated by numerous gaps or fenestrae 100 × 150 nm. across. This thin area of cytoplasm he calls the "sieve plate"; the size of the perforations he suggests is important in governing the passage of chylomicrons of lipid into the perisinusoidal space. A typical basal lamina is absent from the endothelial cells. In consequence injected particulate matter has been shown to appear on both sides of the endothelial cells as soon as five seconds after the injection into the portal vein. The sinusoids pass radially inwards to empty into the central intralobular vein, from which the blood eventually passes to the hepatic veins. Between the

sinusoid endothelium and the parenchymal cells of the liver there is the perisinusoidal *space of Disse* (Fig. 21.10). For many years the genuine nature of these spaces was doubted but E/M studies have shown that the endothelium rests on the tips of numerous small microvilli of the parenchyma cells (Fig. 21.10). The perisinusoidal spaces are believed by some to be the initial part of the lymphatic system in the liver; they almost certainly contain plasma rather than interstitial fluid and are not lined by endothelial cells as are true lymphatics. This direct access of blood plasma to the surface of the hepatic cell is certainly of great functional significance when one considers the very active exchange of metabolites between liver and blood stream which is constantly taking place. It is true, however, that more lymph drains away from the liver than from any other organ in the body and the spaces of Disse may be of significance in explaining this fact. The intralobular veins unite to form sublobular veins which in turn unite to form the hepatic veins. It must be remembered that the sinusoids also receive oxygenated blood directly from the tributaries of the hepatic artery. Intralobular capillaries arising from this latter may empty into either the more peripheral or into the deeper sinusoids of the lobule.

Connective Tissue

In the human liver the connective tissue of Glisson's capsule (see p. 316) is small in amount, but it continues into a dense network of fine reticular fibres round the sinusoids, keeping them patent and supporting the hepatic cells. There is no connective tissue actually between the hepatic cells.

Nerves

The nerves are chiefly post-ganglionic sympathetic fibres and parasympathetic fibres from the vagus which enter the hepatic tissue via the coeliac ganglion. They run with the blood vessels and bile ducts and form plexuses round them. The terminal fibrils supply the blood vessels and the bile ducts.

Functions

The liver is the largest organ in the body (about 1500 gm. in an adult) and is essential for the processing of the food stuffs absorbed from the alimentary canal; all the reactions involved in intermediary metabolism occur in the liver. In particular, the liver is concerned with

(i) the storage of glycogen and glycogenolysis by phosphorylation to maintain the normal blood glucose concentration;
(ii) transport of lipids by transformation of the absorbed triglyceride into the portable lipoproteins;
(iii) the synthesis of the plasma proteins;
(iv) the interconversion of amino-acids;
(v) the deamination of proteins and the production of urea from the end products of nitrogen metabolism;
(vi) the formation of bile salts and bile pigments;
(vii) vitamin storage and metabolism;
(viii) the inactivation of steroid hormones and the detoxification of drugs.

Variations in Structure under Different Conditions

(1) **Varying Age.** In the developing foetus the liver is one of the chief blood-forming organs, haemopoiesis occurring both inside and outside the vascular wall. This process ceases at about the seventh foetal month, but the potentiality remains, and in circumstances of great need haemopoiesis may again occur.

In old age atrophy is common, and if this is associated with an increase of pigment the condition is spoken of as "brown atrophy".

(2) **Varying Diet and Varying Functional Activity.** By the usual histological techniques both fat and glycogen are dissolved. By using appropriate methods both these substances can be demonstrated in the hepatic cells in reciprocal amounts. After absorption of carbohydrate the cells are filled with glycogen granules: if much fat is included in the diet the hepatic cells contain large fat droplets. In starvation, at first the glycogen diminishes, and later the cells contain much fat (fatty infiltration) which has been mobilized from the fat depots elsewhere for use during the glycogen shortage.

(3) **Circulatory Disturbances.** Obstruction of the *portal vein* does not alter the structure of the liver very materially. Obstruction of the *hepatic vein* produces an increase of back pressure in the sinusoids and consequently great vascular engorgement, which is at first most marked near the centre of the lobules, and results in pressure atrophy of the liver cells. This result follows also in the chronic passive congestion that is due to interference with the normal circulation, such as occurs in certain cardiac lesions.

Obstruction of the *hepatic artery* causes necrosis due to ischaemia.

(4) **Bile Flow Disturbances.** If the outflow of bile is prevented (e.g. by calculus in the ducts, sclerosis of the liver constricting the ducts, inflammatory blockage of ducts, etc.) the small bile ducts and capillaries are much dilated: the bile may pass into the blood stream and give the skin the greenish-yellow hue of jaundice.

(5) **Toxic Conditions.** The liver cells are easily affected by toxic conditions of all kinds, as would be expected from a consideration of their functions and of the minute structure of the liver; in particular it has been shown that large increases in the amount of smooth surfaced endoplasmic reticulum occur in the hepatic parenchyma cells after the administration of drugs such as barbiturates and liver poisons, e.g. carbon tetrachloride. This is apparently a response to the need to breakdown the foreign substance by enzymic action.

(6) **Repair after Partial Removal.** Repair occurs rapidly and is accomplished by division of remaining hepatic cells, by enlargement of the cells, and possibly also by differentiation of cells from budding interlobular bile ducts.

THE GALL-BLADDER AND BILE DUCTS (Fig. 21.12)

The lining of the gall-bladder is usually thrown into large folds, most of which disappear when the viscus is distended. The wall consists of four coats.

(1) An outer **serous coat** covering that part of the organ not attached to the liver: this consists of reflected peritoneum, and is continuous with that covering the liver.

(2) An outer **connective tissue coat,** consisting of the typical elements and containing the blood vessels, lymphatics and nerves supplying the gall-bladder, and frequent lymphoid nodules.

(3) A **muscular coat,** consisting of irregularly arranged bundles of smooth muscle, with a considerable amount of connective tissue between them. The small amount of muscle is in part the cause of the inability of the viscus to empty itself completely.

(4) A **mucous layer** lining the lumen, thrown up into small folds which branch and anastomose, giving a reticulated appearance to the surface. Small mucosal diverticula are often found, extending through the muscular layer. The epithelial cells are tall and columnar, with a microvillous border and containing lipid and mucin droplets; the nuclei of these cells tend to be basal.

The E/M has shown that in addition to the microvilli, these cells have a very well-developed junctional complex system between the luminal borders of adjacent cells. There are also complex folds projecting from the lateral surfaces of the cells into the intercellular space. Both of these seem to be adaptations for the active transport of water across the cells as the bile is concentrated.

No goblet cells are present and the underlying stroma of connective tissue is very scanty.

In the neck of the gall bladder there are simple mucus-secreting glands lying in the deeper coats with their ducts opening into the lumen.

Extra-hepatic Bile Ducts

There are three main ducts; the *common hepatic* duct from the liver joins with the *cystic duct* from the gall bladder to form the *common bile duct* which opens into the duodenum. Bile is produced by the liver and leaves by the common hepatic duct. Because of the contraction of the sphincter at the base of the bile duct the secretion has to pass reflexly up the cystic duct

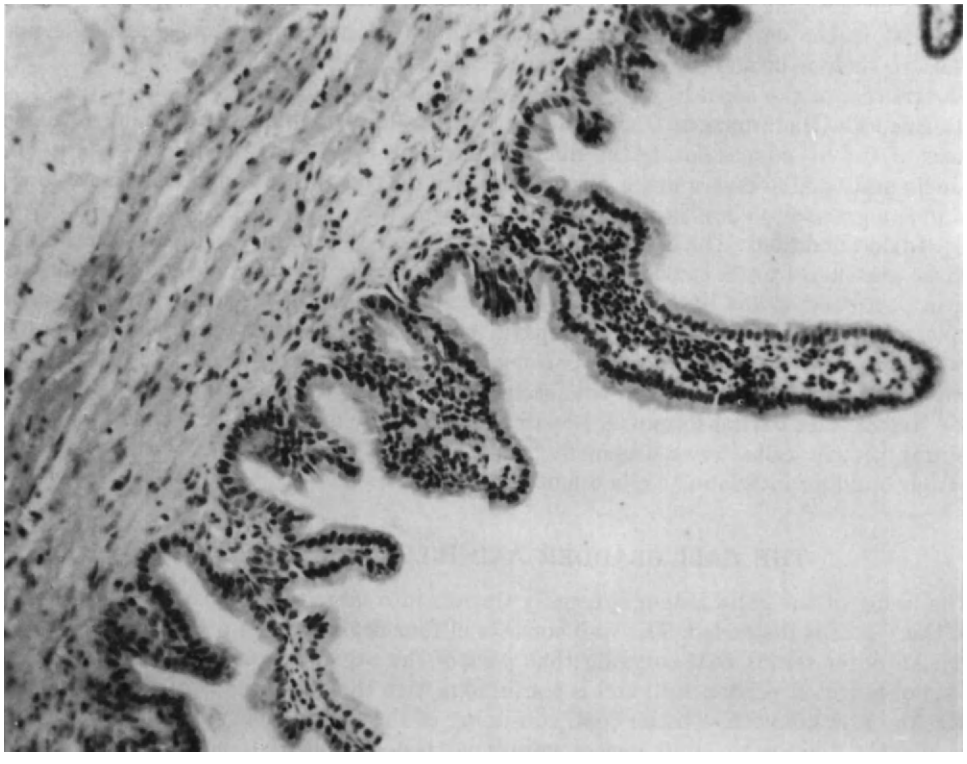

FIG. 21.12. A micrograph of part of the wall of the gall bladder, showing the mucous coat with its columnar epithelial cells and the layer of smooth muscle and connective tissue beneath the mucosa. The lumen of the gall bladder is to the bottom right of the picture. 60 μm.

into the gall bladder where it is stored and at the same time concentrated by the absorption of water. After a meal the sphincter of the bile duct relaxes and the gall bladder contracts so that the bile then passes into the duodenum. In its course to the duodenum the bile duct passes obliquely through the wall of this viscus, where it lies alongside the pancreatic duct; fibres from the duodenal muscle surround the ducts as the *sphincter of Oddi*, while some fibres make a sphincter for the bile duct only. These ducts all have a similar structure. The mucous layer is lined by columnar epithelium: the underlying connective tissue is thick and fibrous, and contains many mucous glands: the whole coat is thrown up into folds. Outside are bundles of muscle, mostly circular in the hepatic duct, and mostly longitudinal in the cystic and bile ducts. Ganglia lie between the muscle and the connective tissue.

Blood Vessels and Lymphatics

The larger blood vessels are found in the connective tissue coat, their smaller branches supplying the muscular and mucous layers. The lymphatic supply consists of two plexuses, a somewhat scanty one beneath the epithelium and another in the connective tissue coat, the latter receiving tributaries from the liver: they drain through the lymph nodes, which are particularly numerous at the neck of the gall-bladder.

Nerves

Post-ganglionic, non-myelinated sympathetic fibres are present, and also pre- and post-ganglionic parasympathetic (vagus) fibres: ganglion cells are found in the connective tissue coat, and sensory nerve endings in the mucous coat. Overdistension or spasm of the gall bladder may stimulate these nerve endings and cause pain, possibly together with disturbances of respiration and reflex disturbances of gut movements.

Functions

The gall-bladder serves as a reservoir for the bile produced by the liver until it is required for discharge into the duodenum. This occurs some time after a meal. The gall-bladder also serves to concentrate the bile within it by active transport of solutes across the epithelial cell luminal border, into the cell and then out into the interstitial space through the lateral borders of the cell. This in turn causes water to move by virtue of the differential in osmotic gradient from the bile into the cell and then into the matrix.

The expulsion of the bile from the gall-bladder is due to the contraction of the gall-bladder musculature. Both nervous and hormonal factors are involved in this emptying process; the hormone *choleocystokinin* which is involved is produced in the duodenal mucosa.

Changes in the Gall-bladder Wall

The gall-bladder is open to infection from the blood stream, from the alimentary canal and from the liver, and consequently it frequently shows pathological change: it is the exception rather than the rule to find the wall of the human gall-bladder normal. The epithelium desquamates very quickly after death.

Obstruction of the bile duct may lead to dilatation with a thinning of the wall. Chronic infection usually leads to a thickening and contraction of the wall, with diminution of the lumen. The presence of gall-stones may produce irritation of the mucous membrane.

THE PANCREAS

The pancreas is a tubulo-alveolar gland whose main duct opens into the duodenum. There is a thin connective tissue capsule which is continued into the substance of the gland as trabeculae that divide the tissue rather incompletely into lobules. The general structure is like that of a serous salivary gland, but with two well-marked differences. Firstly, the secreting alveoli are mostly long and tubular instead of more or less spherical; in a section of the gland therefore relatively few ducts appear to be cut across. Secondly, in addition to the ordinary alveolar tissue, there are scattered through the glandular mass definite groups of lightly-staining epithelial cells without a duct known as the "*islets of Langerhans*" (Fig. 21.15). These are endocrine cells, embedded in the substance of the exocrine gland.

Alveoli

The terminal alveoli consist of granular, somewhat conical cells (the exocrine cells) lining the lumen: their appearance varies with their state of functional activity. When the alveolus is resting the lumen is small and the lining cells are packed with zymogen granules (Fig. 21.13) except for a narrow outer basal zone: during activity the lumen becomes distended

with the secretion and only the innermost part of the cells contains granules. The nucleus is spherical, and nearly always there is an outer zone of cytoplasm between the nucleus and the basal lamina that is clear of granules; this region contains filamentous mitochondria and much highly organized rough surfaced endoplasmic reticulum (Fig. 21.14). This type of ER, found where there is rapid protein secretion for export from the cell, is perhaps seen most characteristically in the pancreatic exocrine cell. There is prominent Golgi apparatus and the zymogen granules may often be seen in electron micrographs as membrane-bounded electron-dense profiles.

In the middle of the alveoli a few spindle-shaped cells can often be made out: these are the continuation of the cells of the long intercalary ducts and are known as centro-acinar cells.

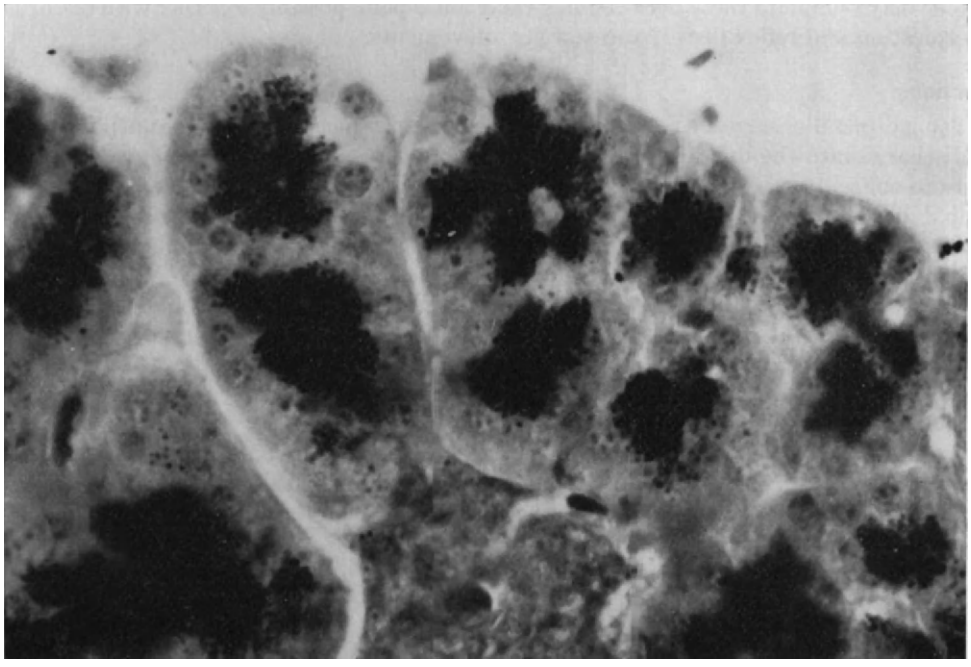

FIG. 21.13. Pancreatic alveoli as seen with the high power of an optical microscope. The preparation has been stained to show the zymogen granules which pack the apices of the cells. 5 μm.

Islets of Langerhans (Fig. 21.15)

These groups of cells are marked off from the alveolar portion by a thin reticular membrane and have an extremely rich blood supply (see Fig. 13.8, p. 183). In the usual section stained with haematoxylin and eosin the cells are pale and appear almost syncytial and devoid of granules (Fig. 21.15). By special staining methods after suitable fixation two types of granule-containing cell may be demonstrated. The α-cells contain rather coarse granules which are coloured red with acid fuchsin and the much more numerous β-cells possess many small granules which stain with orange G or, more specifically, with Gomori's aldehyde fuchsin. These granules in the fresh state are alcohol soluble. In addition there are non-granular indifferently staining cells. In various animal species, other cell types have been described in addition to the α and β cells.

With the E/M the granules appear electron-dense (Fig. 21.16) and are surrounded by a membrane. Often there is a clear zone (which seems likely to be an artifact of preparation) between the granule and the surrounding membrane. The capillaries in the islet tissue are in

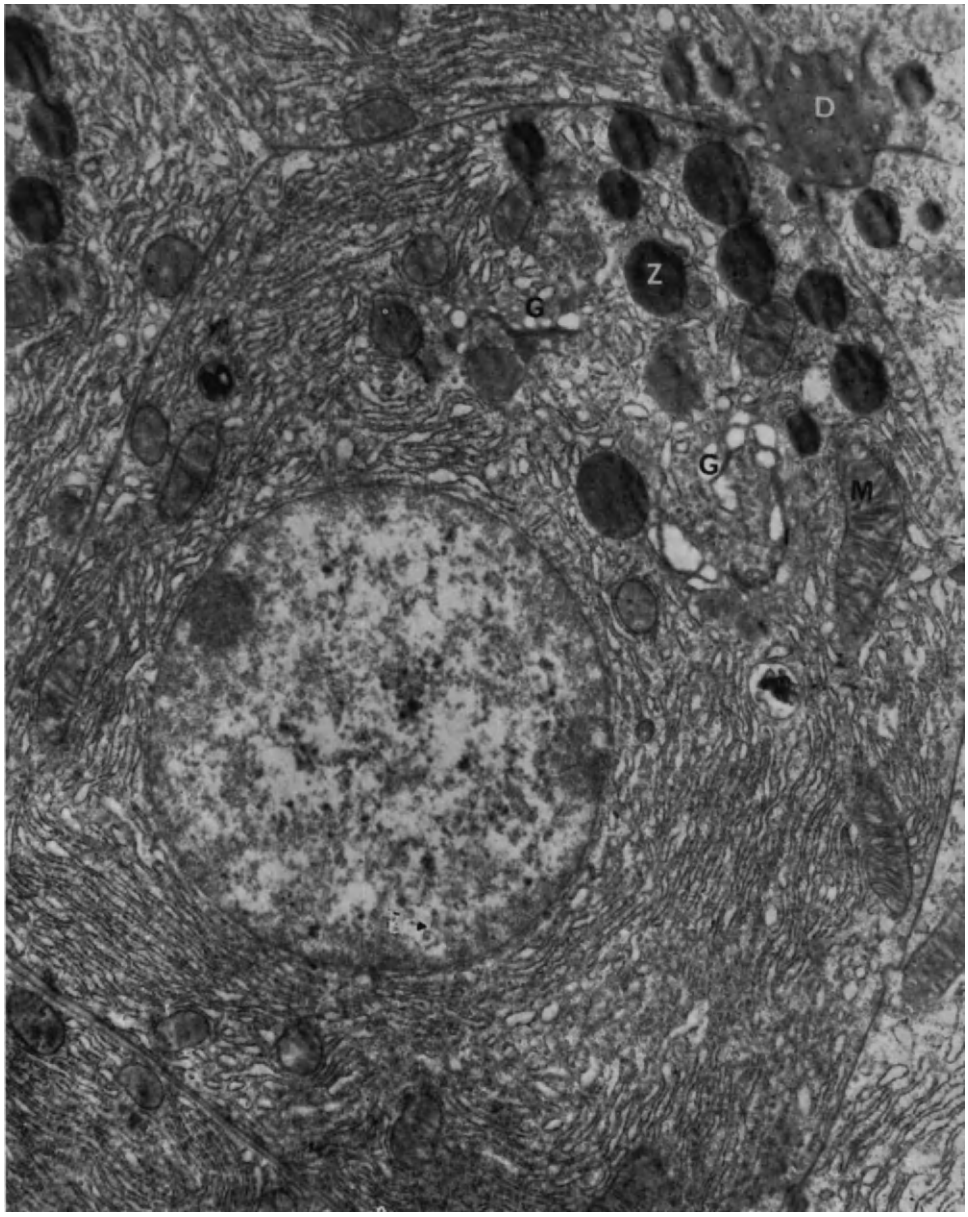

FIG. 21.14. A low power electron micrograph of a pancreatic exocrine cell. The nucleus is clearly visible as well as the mass of rough surfaced ER which fills the cytoplasm. Vesicles of the Golgi apparatus (G), mitochondria (M) and zymogen granules (Z) may also be seen. D represents the lumen of the acinar duct. 1·25 μm.

close contact with the bases of the cells and have endothelial walls which possess numerous fenestrae. This close relationship is doubtless an adaptation for the rapid removal of the insulin and glucagon by the blood stream. There is little doubt that the granules of the β-cells contain the antecedent of the hormone *insulin*, whereas there is evidence suggesting that the α-cells produce *glucagon*. Very rarely an islet can be seen connected by a solid strand of undifferentiated cells with the epithelium of the small ducts from which they are originally developed.

The islets vary very much in size and are more numerous in the tail of the pancreas than elsewhere: it has been calculated that in man there are more than a million islets in the organ.

Ducts

The lumen of the secreting alveoli is lined with the centro-acinar cells which are continuous with the spindle-shaped cells of the intercalary ducts (intralobular ducts): these join together into larger interlobular ducts which finally drain into the pancreatic duct or the accessory pancreatic duct (if present). The intralobular ducts are lined by a low columnar epithelium, while the larger interlobular ducts have a typical columnar epithelium with occasional goblet cells and are surrounded by dense connective tissue.

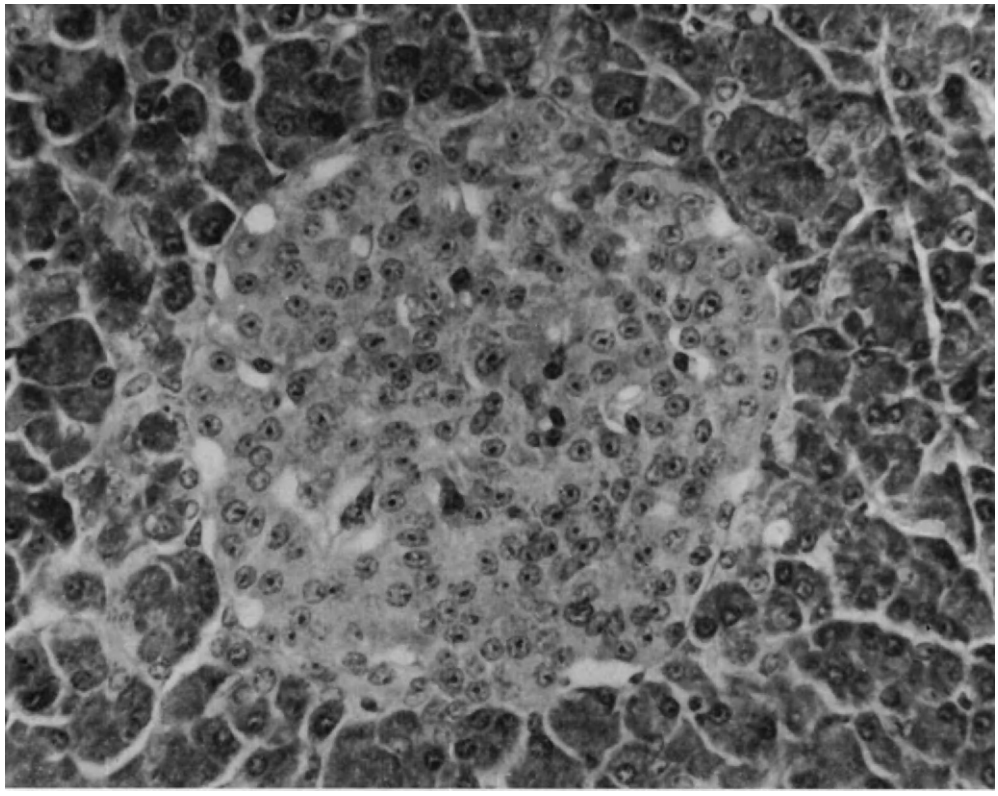

FIG. 21.15. Part of the pancreas to show the appearance in a typical histological preparation of an islet of Langerhans (centre). Note the pale staining of the cytoplasm of the cells and the numerous blood vessels in the islet tissue. 20 μm.

Blood Vessels

The blood supply is very rich, particularly to the islet tissue. The larger vessels run in the interlobular connective tissue. Each alveolus is surrounded by a capillary network outside the basal lamina, and the islet capillaries are large and sinusoidal.

Lymphatics

The distribution of lymph vessels within the organ has not been worked out in detail. Lymph from the pancreas drains into the upper groups of pre-aortic lymph nodes accompanying the splenic artery.

Nerves

The nerve supply is twofold. Myelinated fibres from the vagus are found: these probably arborize in relation to the nerve cells found scattered in the interlobular connective tissue. Non-myelinated, post-ganglionic sympathetic fibres, from the coeliac plexus, enter with the arteries and end by ramifying round the alveolar cells. Some of the fibres pass to the islet tissue. In addition, many non-myelinated fibres supply the blood-vessel walls.

(Pacinian corpuscles are found in the pancreas of cats and of some other animals: this is due to the fact that the mesentery contains these bodies, and that in these animals the pancreas happens to have a small prolongation between the layers of the mesentery.)

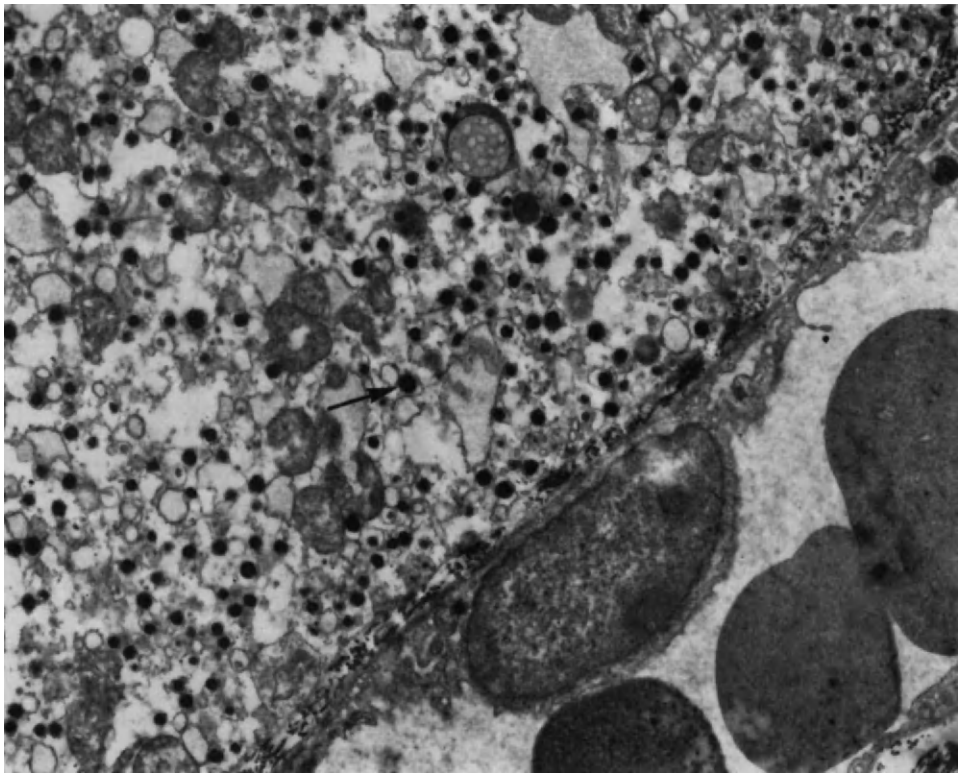

FIG. 21.16. An E/M of the periphery of an islet cell, showing the electron-dense secretory granules (one of them arrowed) of insulin precursor. Note the close relationship with the capillary (lower right); an endothelial cell nucleus is prominent and three red blood corpuscles may be seen in the lumen of the capillary. 1·5 μm.

Functions

The exocrine portion of the pancreas serves to produce pancreatic juice, rich in bicarbonate; this acts largely to neutralize the very acid contents entering the duodenum from the stomach. The production of a pancreatic juice rich in bicarbonate but containing little enzymes is due to the action of *secretin*, a hormone liberated by the duodenal mucosa. A second hormone, *pancreozymin*, is also produced by the duodenum and this latter causes the secretion of large amounts of trypsinogen and chymotrypsinogen, together with lipases and amylases by the exocrine cells of the pancreas. Both types of secretion occur after a normal meal. Stimulation of the vagus nerve will also cause release of the pancreatic enzymes but without the production

of much watery secretion whilst the inhibition of parasympathetic nervous activity by atropine will inhibit the release of the enzymes.

The endocrine portion of the pancreas secretes two hormones; the β-cells produce insulin, whilst the α-cells secrete glucagon. Both of these are concerned in the carbohydrate balance of the body, insulin favouring the conversion of glucose to glycogen whereas glucagon has the opposite effect. In the absence of sufficient insulin, the blood sugar concentration rises rapidly.

Variations in Structure under Different Conditions

After death, the pancreas undergoes autolysis with great rapidity.

(1) **Functional Activity.** The changes in the alveolar cells during functional activity have been mentioned above: prolonged stimulation by secretin, or by the nerves, may almost entirely empty these cells of granules. The islet cells are not affected.

(2) **Diabetes Mellitus.** Although this condition is due to lack of the active principles produced by the islet cells, it is not always possible to detect any microscopic lesion of this tissue. Sometimes a hyaline degeneration is seen, and sometimes there is a fibrosis involving the islets, and sometimes a simple atrophy of islet tissue. In general the β-cells suffer rather than the α-cells.

(3) **Regeneration after Damage.** If the tissue is damaged there is a formation of new islets from the duct epithelium, but usually very little regeneration of alveolar tissue. There is no transition between islet and alveolar tissue, although both are formed from duct epithelium.

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CHAPTER 22

THE URINARY SYSTEM

The urinary system consists of:

(1) The two *kidneys*, the glandular organs whose function is selectively to concentrate and eliminate certain constituents of the blood.

(2) The two *ureters*, the excretory ducts leading away from the pelvis of the kidney.

(3) The *urinary bladder*.

(4) The *urethra*, the duct passing from the bladder to the outside. The male urethra is a duct common to the urinary and genital systems: the female urethra belongs only to the urinary system.

THE KIDNEY

The kidney is a compound tubular gland enclosed in a strong *capsule* of connective tissue that contains a few smooth muscle fibres. In the foetus, septa pass from the capsule into the organ dividing it into lobes (see Fig. 22.1): occasionally this condition persists in the adult producing a "lobulated kidney". On the medial aspect the depression known as the *hilum* marks the entrance of the renal artery and the exit of the renal vein and the ureter.

When cut open longitudinally the kidney is seen to consist of an outer *cortex* and an inner *medulla*: they both contain the uriniferous tubules. The cortex looks granular to the naked eye because the tubules are here much convoluted and it also contains the renal corpuscles: the medulla looks striated as the tubules run through it in a straight course radiating towards the pelvis. In the medulla also are the *medullary rays* where the tubules are running in a straight course. The medulla consists of a varying number (8 to 18) of conical masses, the *renal pyramids* (of Malpighi), whose bases are adjacent to the cortical tissue, and whose apices form the *papillae*: frequently several pyramids fuse to form one papilla. These papillae project into the *pelvis*, which is the expanded beginning of the ureter. (In some animals, e.g. guinea-pig and rat, there is only one pyramid.) The pyramids correspond to the foetal lobes.

Both cortex and medulla consist of closely-packed uriniferous tubules (Fig. 22.4), the amount of interstitial connective tissue being very small in the cortex and rather more in the medulla. The functional unit of the kidney is the *nephron*, this consists of a glomerulus and its tubule (see below): there are said to be about 1,000,000 nephrons in each kidney.

The *uriniferous* tubules seen in any section are cut in many directions. If unravelled by microdissection or examined in a macerated preparation a tubule is found to consist of the following parts (Fig. 22.2):

(1) A dilated blind end, the *glomerular* (*Bowman's*) *capsule*, which is invaginated to enclose a tuft of convoluted blood "capillaries", the *glomerulus*: the whole structure is called a *Malpighian* (*renal*) *corpuscle* (Figs. 22.2, 22.3).

(2) The tubule leaves the capsule by a constricted neck, passing into the greatly convoluted *proximal convoluted tubule* (Fig. 22.2).

(3) The tubule then takes a straight course down to the medulla, where it doubles round and comes up again to the cortex. The loop thus formed is called the *loop of Henle* and is divided into descending and ascending limbs. The descending limb has a squamous epithelium; the ascending limb has in part a squamous epithelium and in part a more cuboidal type. These straight, parallel tubes are found in the medullary rays of the cortex.

(4) The tubule leaves the medullary ray and enters the cortical tissue again, becoming the *distal convoluted tubule*: it lies near its own Malpighian corpuscle (Fig. 22.2).

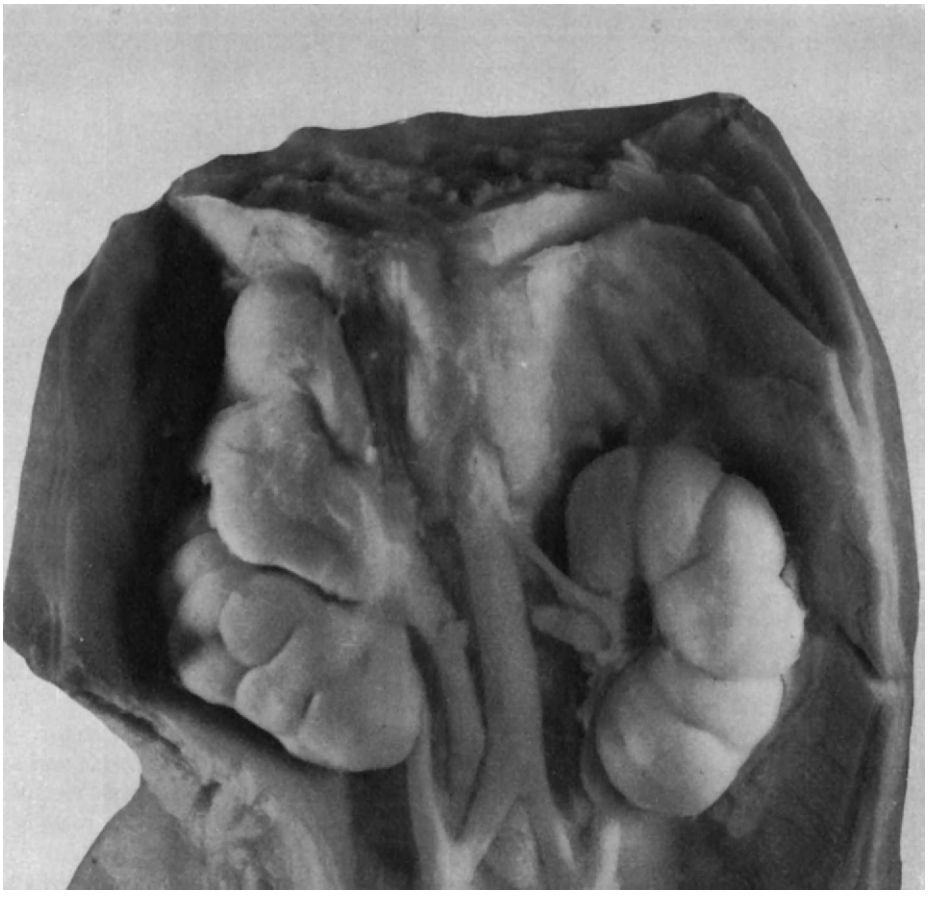

FIG. 22.1. A photograph of the posterior abdominal wall of a human foetus to show the lobulated nature of the large kidneys.

0·5 cm.

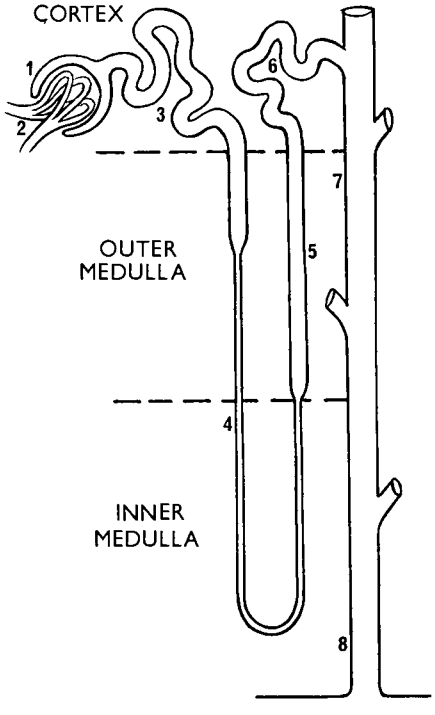

FIG. 22.2. A diagram of the organization of the nephron. 1, glomerular capsule; 2, glomerulus; 3, proximal convoluted tubule; 4, thin part of the loop of Henle; 5, thick part of the loop of Henle; 6, distal convoluted tubule; 7, collecting duct; 8, papilla.

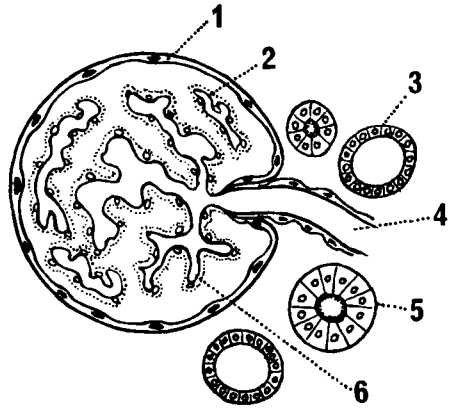

FIG. 22.3. A diagram of the glomerulus and associated structures; 1, parietal epithelium: 2, capillary network; 3, distal convoluted tubule; 4, efferent arteriole; 5, proximal convoluted tubule; 6, basal lamina of glomerular capillary. The connection of the urinary space with the tubule of the nephron is not shown in this diagram.

(5) The tubule then straightens out again, and by a short *junctional tubule* joins a straight *collecting tubule* which passes down in a medullary ray, to enter a medullary pyramid, receiving other junctional tubules on the way: finally a large straight tubule, the *papillary duct* (*of Bellini*), opens into the pelvis at the apex of the papilla.

The wall of the uriniferous tubule consists of a single layer of epithelial cells that rests on a well-marked basal lamina which is very closely connected with the interstitial reticular tissue.

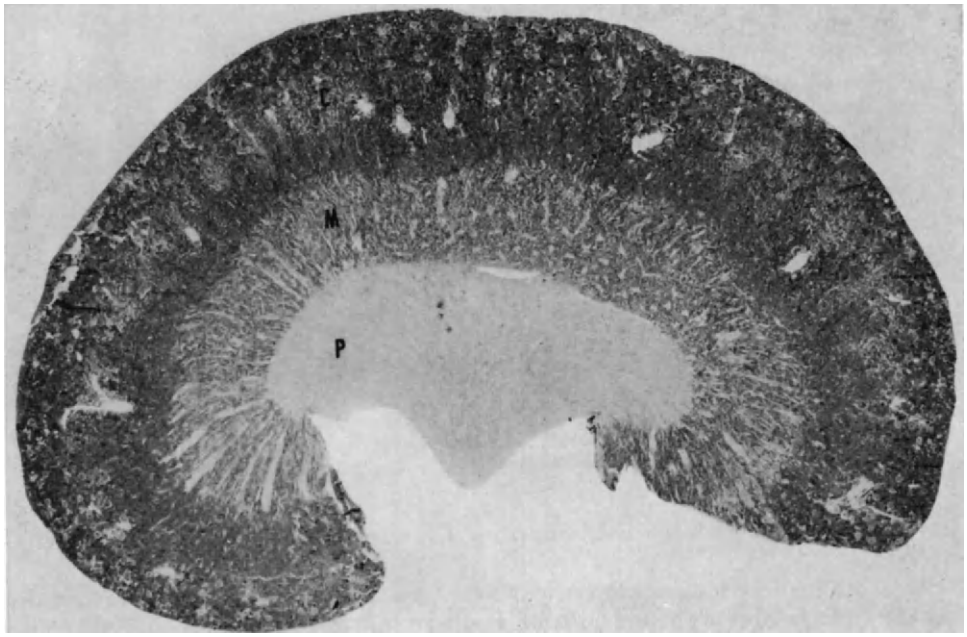

FIG. 22.4. A longitudinal section of the unipyramidal kidney of a rat. The glomeruli may be just seen at this magnification in the cortex (C); the medulla (M) and the pyramid (P) may also be seen. 2 mm.

Renal corpuscle (Malpighian corpuscle) (Figs. 22.5, 22.6)

The renal corpuscle is essentially a two-layered cup of squamous epithelium enclosing a space, the *urinary space*, which is continuous with the lumen of the remainder of the nephron. The renal corpuscle is invaginated during the course of development by the vascular tuft or

glomerulus. The parietal epithelium of the renal corpuscle remains essentially squamous, whilst the visceral layer in contact with the invaginated capillaries becomes modified to form what are termed *podocytes* (Figs. 22.5, 22.6). The podocytes are cells which have their bodies separated from the basal lamina with which they remain in contact only by extensions of their cytoplasm known as *foot processes*. The cell body of the podocyte lies free in the urinary space. Adjacent foot processes of the podocytes are separated from each other by thin spaces known as *filtration slits* (Fig. 22.6) or slit pores; in good electron micrographs these are often seen to be closed by means of a thin dense membrane, the *slit membrane* (Fig. 22.6B). These

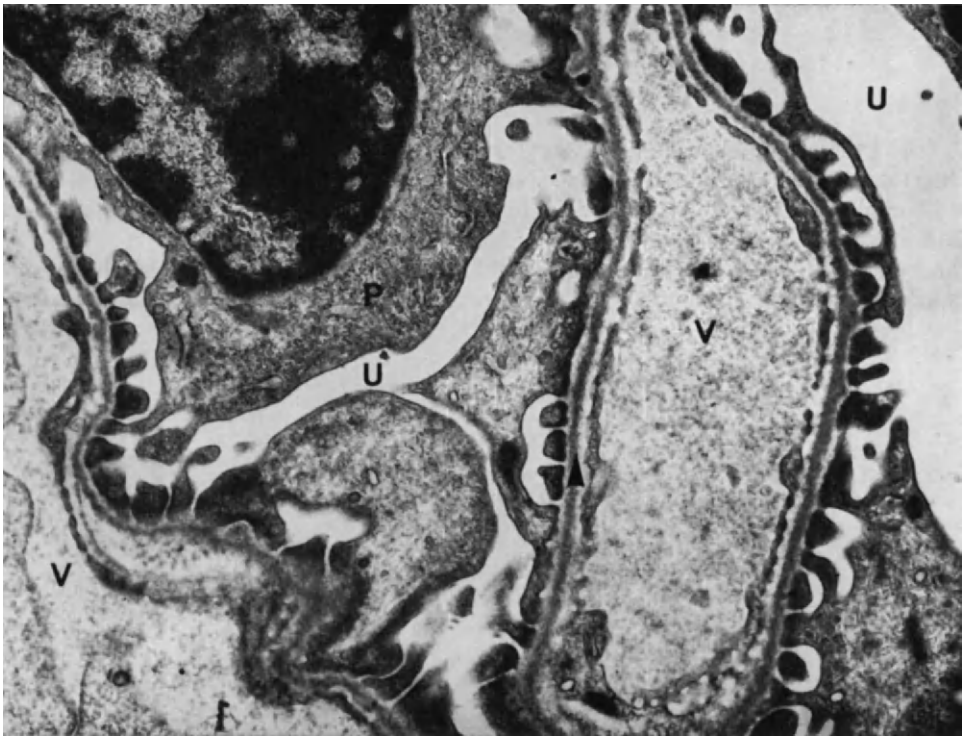

FIG. 22.5. A low power E/M of part of a glomerulus. P, the cell body of a podocyte with its nucleus and foot processes; U, urinary space; V, lumen of vessel. The basal lamina is indicated by a small arrow.

cells have much rough surfaced ER, numerous mitochondria and a well developed Golgi apparatus.

The basal lamina in the renal corpuscle is well developed and serves in common with that covering the endothelium of the invaginated capillary tuft of vessels. It is often thought that the vessels in the renal corpuscle are arranged in loops but it seems more likely that they form an anastomosing and branching network. The endothelial cells of the glomerulus are very richly fenestrated with larger pores than found in other sites and with pores which appear to lack any trace of a closing diaphragm. Thus it seems that the only continuous layer between the blood and the urinary space of the nephron is the basal lamina. Passage of substances across this is by filtration; as there is a pressure of about 70 mm. Hg in the glomerular capillaries, after taking into account the opposing colloid osmotic pressure of the plasma and the pressure inside the urinary space, there will be a differential of about 25 mm. Hg to effect glomerular

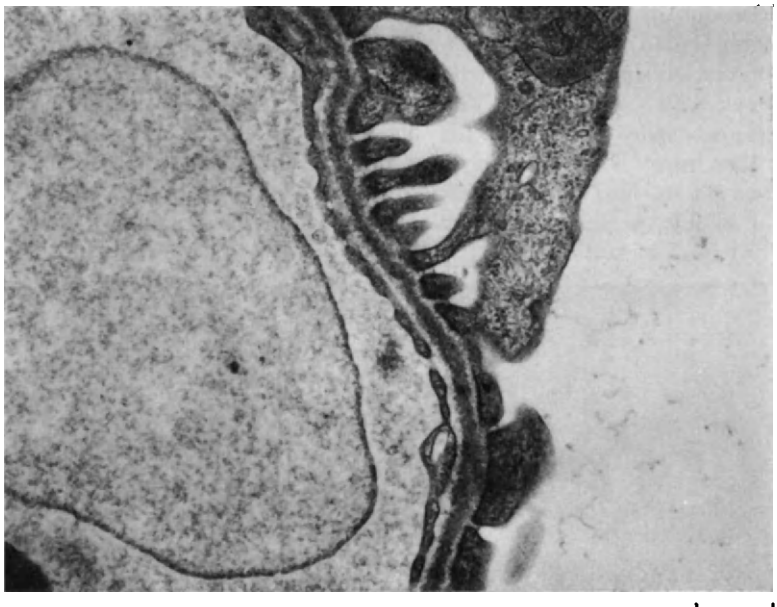

FIG. 22.6A. E/M of part of a glomerulus with the vascular lumen on the left containing a red blood corpuscle; podocyte foot processes are at the upper right and the urinary space on the extreme right. Note the marked fenestrae in the vascular endothelium. 1 μm.

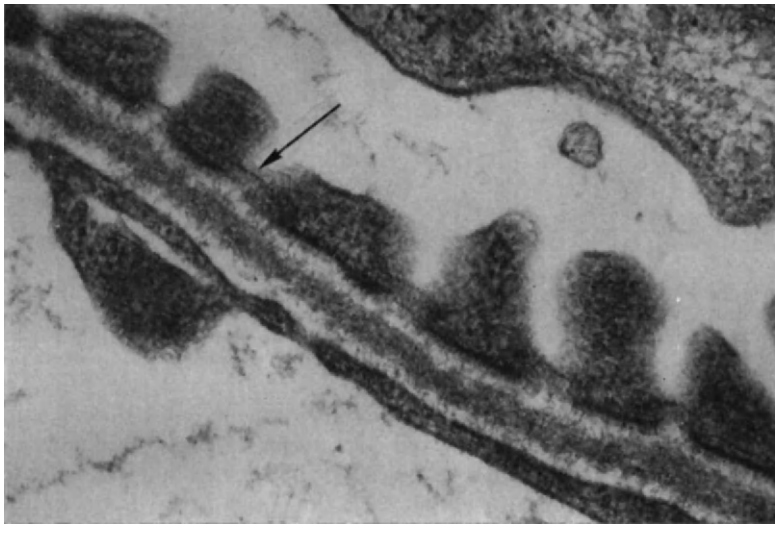

FIG. 22.6B. High power E/M of the barrier between the vessel and the urinary space. The vascular endothelium is at the lower left, the basal lamina and the podocyte foot processes on the diagonal of the picture. The arrow indicates one of the filtration slit membranes. 0·5 μm.

filtration. The glomerular filtrate is essentially similar to plasma but lacks the larger molecules in the latter. Control of the molecular size in the glomerular filtrate may be due to:

(i) the composition of the basal lamina, which acts as a "molecular sieve", rather in the same manner as an ion exchange resin (Huang *et al.*);

(ii) the texture of the basal lamina; this normally is permeable only to molecules less than 100 Å in diamcter (Latta);

(iii) to the filtration slit size. Some evidence that this is important is provided by the work of Karnovsky and his collaborators. They used small horseradish peroxidase and large myeloperoxidase molecules as markers for the electron microscope. They suggest that small molecules can pass into the urinary space, whereas large molecules can only pass through the basal lamina but get stopped by the filtration slits.

The volume of filtrate produced by the glomeruli in the kidney has been estimated at 200 litres per day in a normal human.

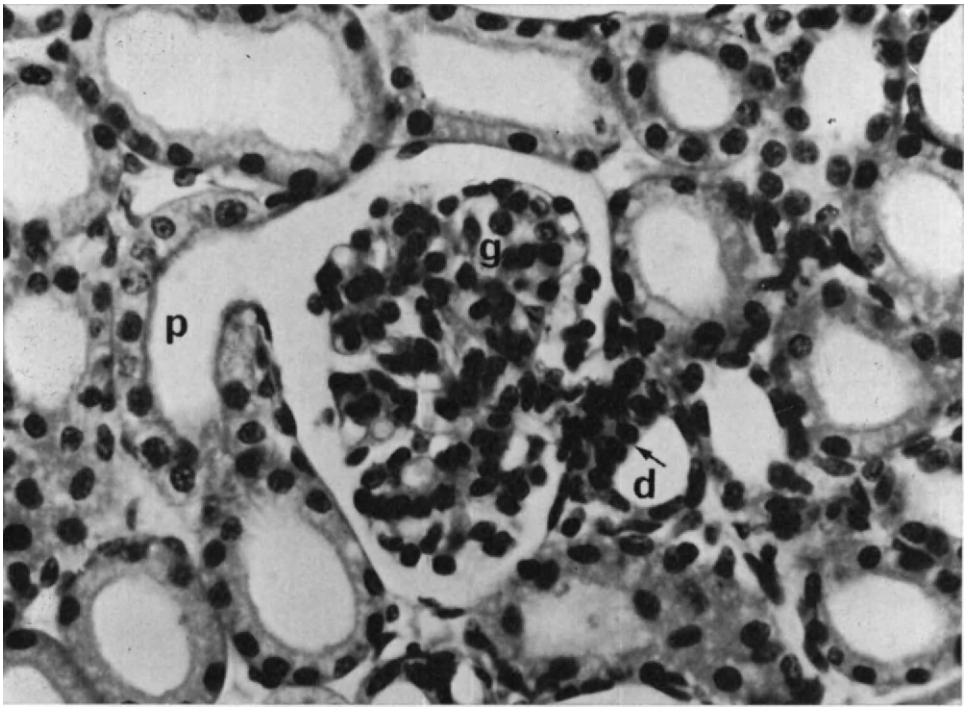

FIG. 22.7. A micrograph of the glomerulus and associated structures. The glomerular capillary tuft (g) may be seen invaginating into the urinary space which here is seen to be continuous with the proximal part of the convoluted tubule (p); d is part of the distal convoluted tubule, the arrow indicates the prominent nuclei of the macula densa. 15 μm.

The smooth muscle cells in the wall of the afferent arteriole just before it enters the renal corpuscle and breaks up into the capillary tuft are modified. They become rounded and are characterized by the presence of numerous small cytoplasmic granules. These are the *juxtaglomerular granules* which are closely related to a thickened area of the wall of the distal convoluted tubule which is in contact with the afferent arteriole. This area of the tubule is known as the *macula densa* (Fig. 22.7). The juxtaglomerular granules may be demonstrated by specific staining techniques (Fig. 22.8) and various elegant experimental techniques have shown that they contain the vasopressor substance *renin*. This, acting via the intermediates angiotensin I and angiotensin II, probably plays an important part in regulating the release of aldosterone by the adrenal cortex and so controlling increased sodium retention by the kidney. The cells of the macula dcnsa often show changes in their enzyme histochemistry when the rate of secretion of thc juxtaglomerular granules is alterecd, so that they are often regarded as being complemcntary to the juxtaglomerular cells of the arteriole wall, e.g. by acting as osmo-

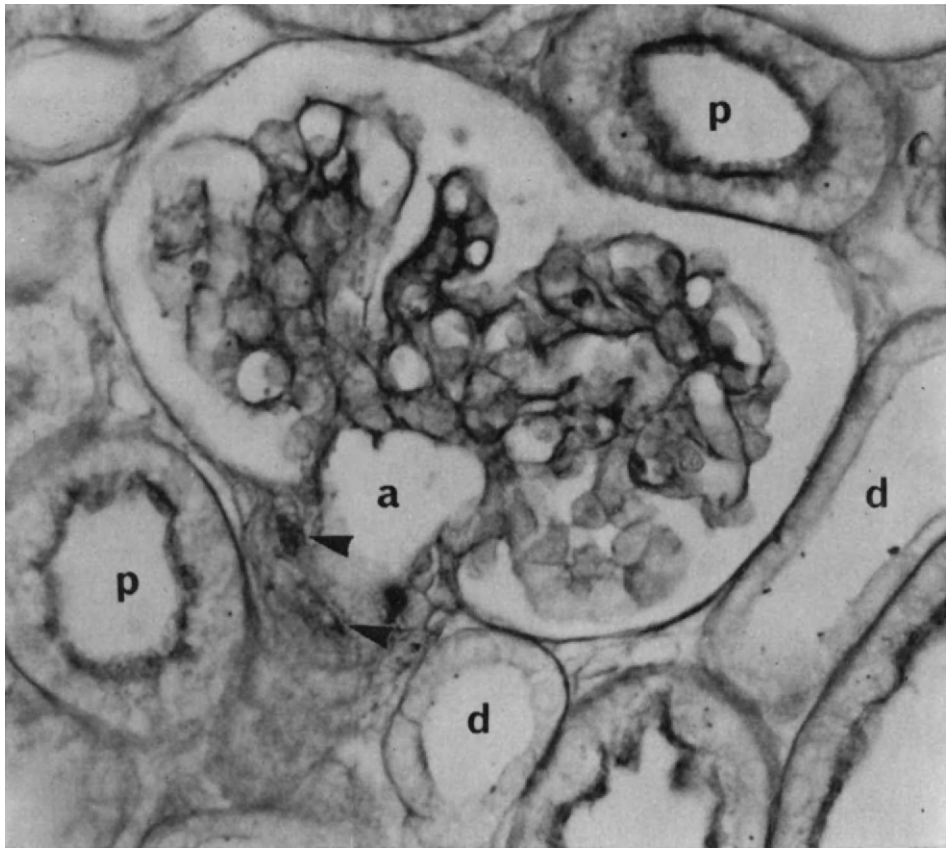

FIG. 22.8. A micrograph of a glomerulus and the associated structures stained with the PAS technique for mucopolysaccharides. The basal laminae of the tubules and glomerulus stain heavily with this technique as do the juxtaglomerular granules (arrows) and the striated border of the cells of the proximal convoluted tubule. p = proximal convoluted tubule; a = afferent arteriole of the glomerulus; d = distal convoluted tubule.

10 μm.

receptors; the whole structure is often termed the *juxtaglomerular apparatus*. The cells of the macula densa possess fewer mitochondria and fewer infoldings of the basal plasma membrane than the typical cell of the distal convuluted tubule.

Proximal Convoluted Tubule (Fig. 22.9)

This part of the nephron is about 40–60 μm. in diameter and is continuous with the renal corpuscle (Fig. 22.7); the proximal tubule is characterized by its tortuous nature, occupying much space in the kidney cortex before turning inwards towards the medulla as the *loop of Henle*. The proximal convoluted tubule is lined by a typical columnar epithelium which, when visualized by the optical microscope, shows the cells with a prominent striated border at their luminal aspect. They may also show faint striations at their base in material which is well fixed. The E/M shows that the luminal border is composed of very long, regular microvilli (Fig. 22.10) which are very closely packed together. Again if the material is well fixed there is a prominent lumen to the tubule but if the preservation is (as is often the case) inadequate, then the proximal tubules often appear to possess very little lumen and the microvilli of the opposing edges of the tubule may even be in contact with one another.

There is usually a well marked Golgi apparatus in the cell, prominent lysosomes and often vacuoles in the apical cytoplasm. Many lateral cell interdigitations are present and the basal plasma membrane is thrown into elaborate furrows between which many mitochondria are orientated with their long axes parallel to that of the cell. It is these which are responsible for the striations which are visible with the optical microscope.

The cells of the proximal convoluted tubule are known to be responsible for the reabsorption of approximately 85 per cent of the sodium chloride and water of the glomerular filtrate. Most of the glucose in the filtrate and the amino-acids and proteins of small molecular weight are also reabsorbed in this part of the nephron. Certain organic bases and acids (e.g. creatine and para aminohippuric acid) are secreted into the tubule lumen by the cells of this part of the tubule.

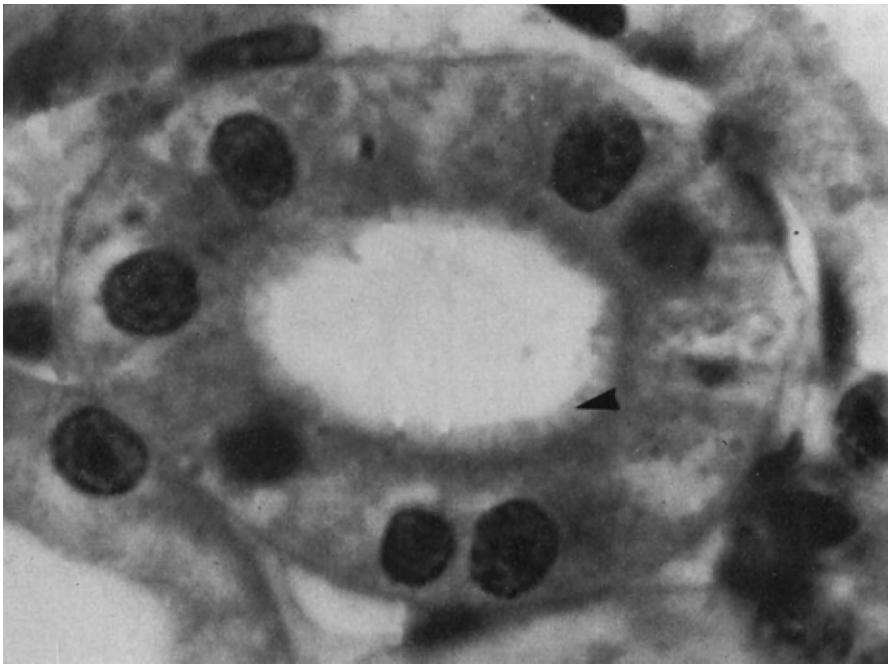

FIG. 22.9.—A micrograph of a single proximal convoluted tubule in transverse section. The arrowhead indicates the prominent "brush border" which is due to the presence of microvilli on the apical surface of the cells. 5 μm.

Loop of Henle (Figs. 22.2, 22.11, 22.12)

This portion of the nephron is described as comprising a descending and an ascending limb; it begins at an abrupt narrowing of the proximal convoluted tubule to form the thin tube (15 μm. diameter) of the descending limb. This runs from the cortex of the kidney down into the medulla and then continues upwards again as the ascending limb. At the junction of the inner and outer regions of the medulla the ascending limb of the loop changes abruptly again to form the thick portion of the loop. The squamous epithelium with nuclei bulging into the lumen which characterizes the thin portion changes here to a cuboidal type of epithelium with many basally arranged mitochondria. The E/M (Fig. 22.12) shows that the loop of Henle has cells with only a very few short stubby microvilli and in the thin segment, many parts of cells are seen in any one section, giving a rather characteristic appearance.

The glomerular filtrate which has entered the loop from the proximal tubule has been

reduced in volume by about 80 per cent but it is still isotonic with the blood. Due to the presence of the thin segment of the loop, the mammal is able to create a hypertonic medium in the interstitial medullary tissue and so produce a hypertonic urine, thus conserving water. The loop cells are able to produce a hypertonic environment in the interstitial tissue of the kidney medulla due to the differing permeabilities of the loop cells and to their differing capacities for active transport. The cells of the ascending limb of the loop of Henle are believed to be impermeable to water but are thought instead to be very effective in the active transport of sodium ions from the urine into the interstitial tissue. This will cause an increase in the osmolarity of the interstitial tissue, thus creating an osmotic gradient from the cortex

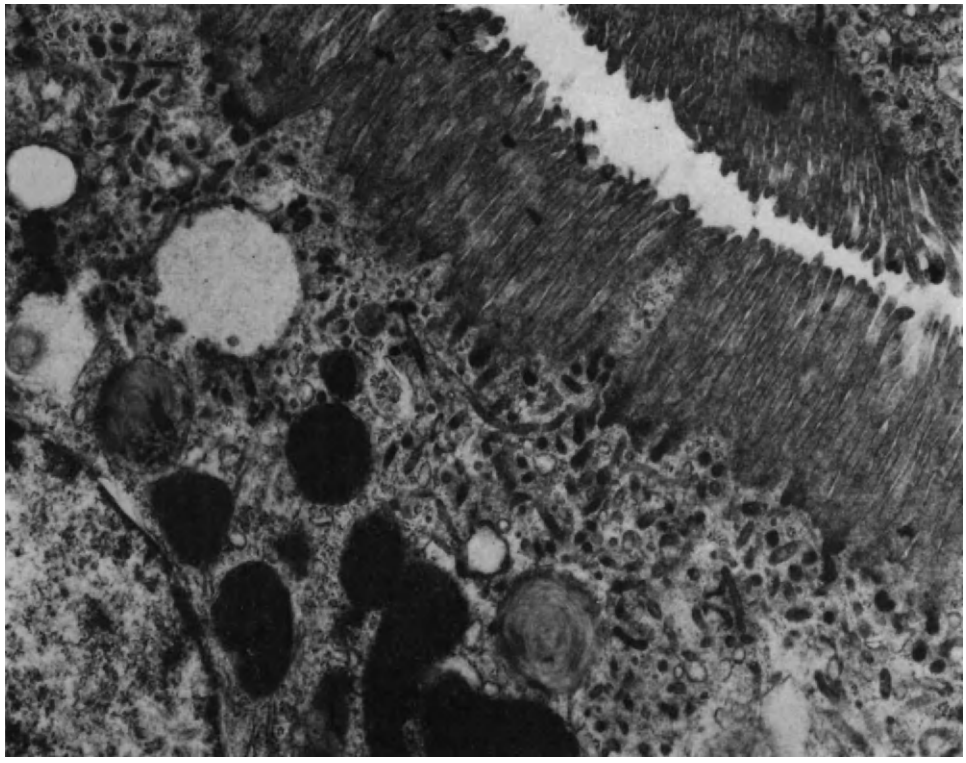

FIG. 22.10. E/M of part of the apical cytoplasm of a cell from the proximal convoluted tubule, showing the prominent microvilli on the luminal border. The dark objects in the cytoplasm are mitochondria; vesicular elements of the smooth surfaced endoplasmic reticulum and vacuoles are also present. 0·5 μm.

which is isotonic with the blood) to the tip of the papilla deep in the medulla (where the nterstitial tissues are considerably hypertonic to the blood). Water therefore tends to leave the descending segment of Henle's loop by a passive diffusion, so making the urine as it moves towards the tip of the loop very hypertonic. At the same time a certain amount of sodium ions enter the descending limb of the loop by diffusion. The blood vessels surrounding the loops (the vasa recta) serve as counter-current exchange systems and allow osmotic equilibration of blood in the two limbs of the vasa recta so that there is no tendency to disrupt the osmolar gradient established between the cortex and the medulla. The sodium pump in the cells of the ascending limb of the loop results in the urine reaching the top of the loop becoming hypotonic to the plasma, although it is now very much reduced in volume. Final adjustment to the tonicity of the urine occurs during its passage through the collecting ducts.

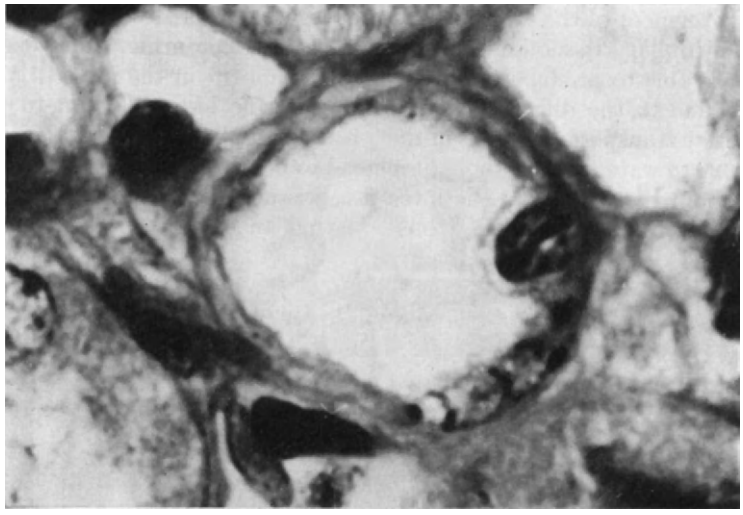

FIG. 22.11. A section through the medulla of the kidney. A thin segment of a loop of Henle occupies the centre of the picture, surrounded by sections of vasa recta. 4 μm.

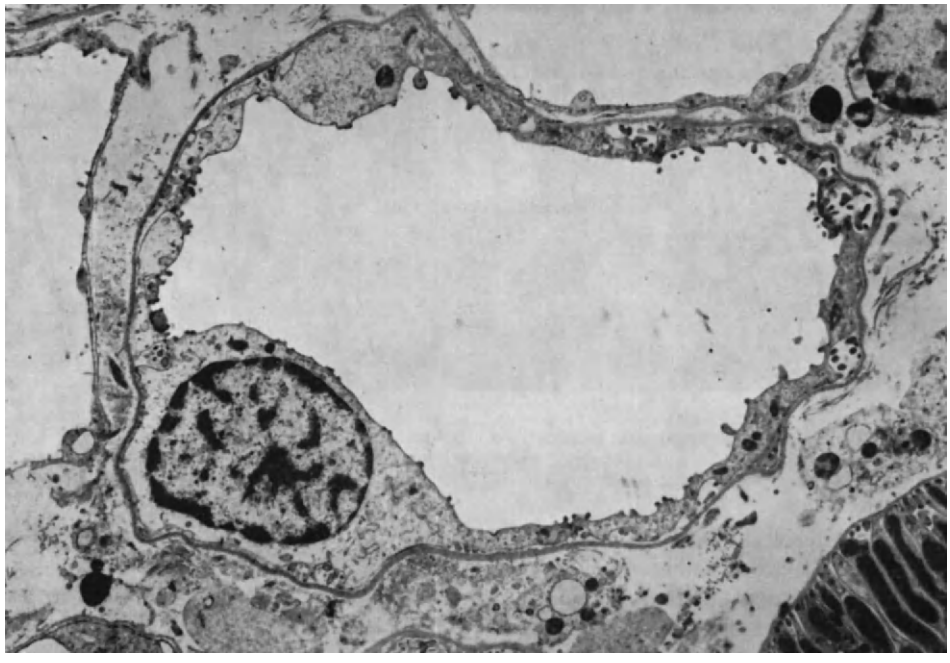

FIG. 22.12. An E/M of a thin segment of a loop of Henle. The nucleus of one of the cells is seen bulging into the lumen. Note that there are only very short microvilli on the cells and that the endothelium of two of the vasa recta (on the left and at the top of the micrograph) is of extreme thinness. 2 μm.

Distal Convoluted Tubule (Fig. 22.13)

The distal convoluted tubules are found in the kidney cortex in close relationship to their renal corpuscle and proximal convoluted tubule. They are about 20–50 μm. in transverse section and can usually be recognized in section under the optical microscope by their lumen which is much more prominent than that of the proximal tubule, by their lack of any distinct "brush border", and by their pronounced acidophilia.

The E/M shows that the cells of the distal convoluted tubule have small microvilli on their luminal surface but their most characteristic feature is their extensive basal plasma membrane infoldings, between which many mitochondria are arranged (Fig. 22.14). This is typical of cells which are involved in the active transport of ions across their cell membrane. In the distal

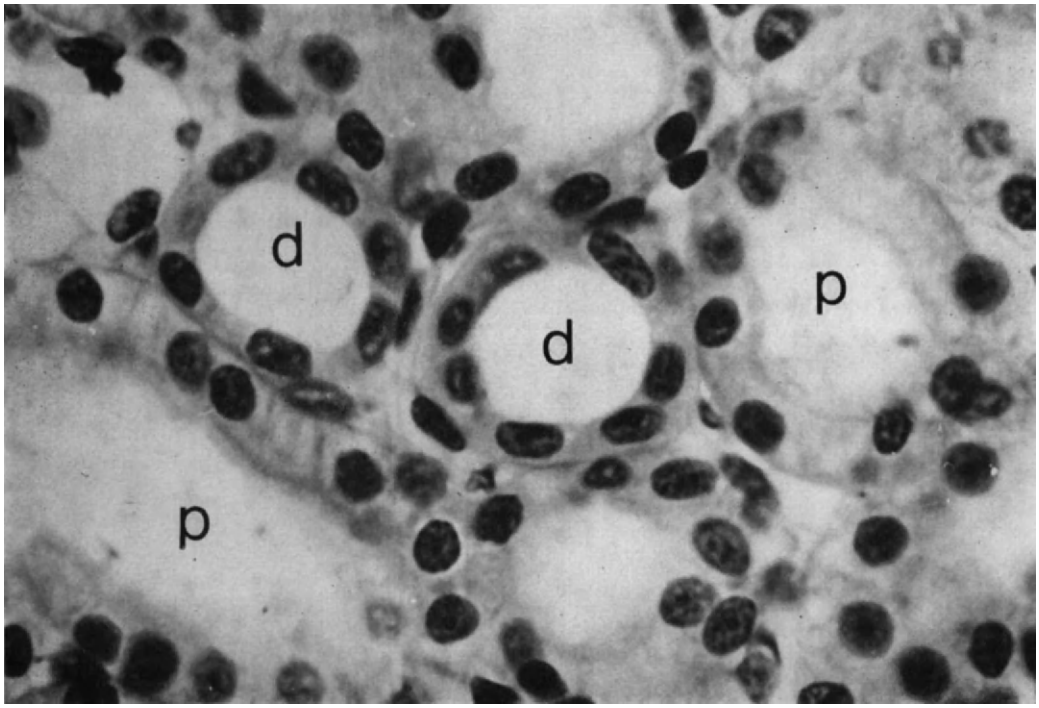

FIG. 22.13. An optical micrograph of part of the kidney cortex showing distal convoluted tubules (d) amongst proximal convoluted tubules (p). The lumen of the distal tubules is often more marked and their cells do not have a "brush border". 10 μm.

convoluted tubule the process of reabsorption of sodium ions from the urine continues but this region is typified by being the principal site at which other cations such as potassium and hydrogen are added to the urine, as well as ammonia. It may be taken therefore that the distal convoluted tubule is the region where the urine is rendered acid.

Collecting Ducts (Fig. 22.15)

The distal convoluted tubule leads via an arched junctional tubule into a straight collecting tubule located in the medullary ray. These straight tubules lie side by side and make up much of the pyramids, before fusing with other collecting tubules in the medulla to form the progressively larger papillary ducts (of Bellini) which open direct into the pelvis of the kidney.

The straight collecting ducts are about 40–50 μm. in diameter, which increases as they near the papillae to a diameter of about 200 μm. The lining epithelium is cuboidal and in the optical

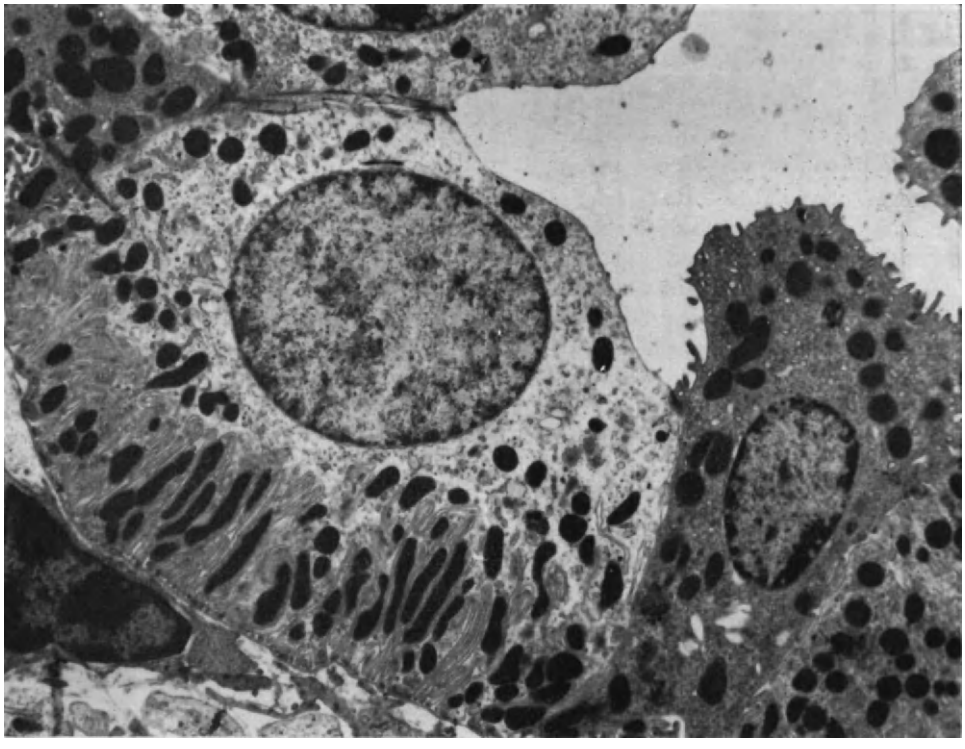

FIG. 22.14A. An E/M of a distal convoluted tubule cell. Note the regular arrangement of the mitochondria at the base of the cell and the absence of microvilli. 1·5 μm.

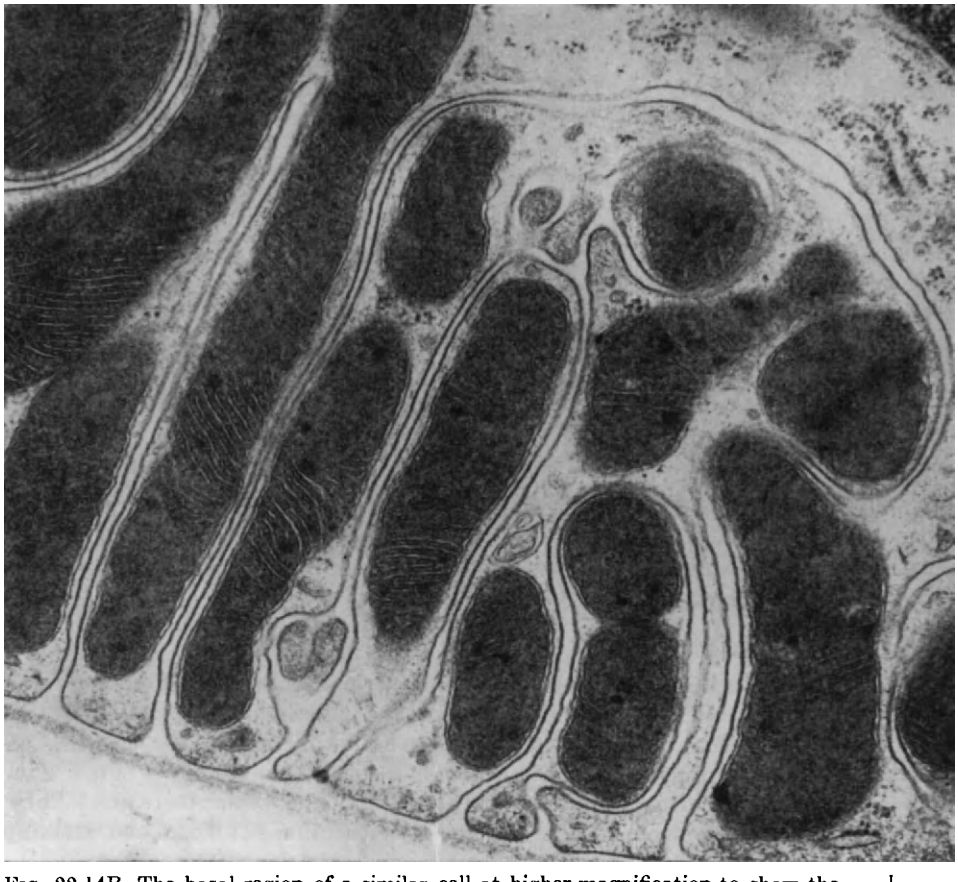

FIG. 22.14B. The basal region of a similar cell at higher magnification to show the basal infoldings of the plasma membrane. Mitochondria are arranged in close association with these membranes. The basal lamina is at the bottom of the picture. 0·1 μm.

microscope the cells show very distinct cell boundaries (Fig. 22.15). The E/M shows (Fig. 22.16) that these are "light" cells with very few cytoplasmic organelles; there are very prominent lateral and basal infoldings of the plasma membrane (Fig. 22.16).

The collecting ducts pass radially from the cortex into the medulla, i.e. from an area of isotonicity to an area which is greatly hypertonic to the blood. If there is no antidiuretic hormone (ADH) from the pituitary circulating in the blood stream, then the cells of the collecting duct are impermeable to water and the urine passing through them remains hypotonic and dilute. If, however, there is ADH in the blood then the cells of the walls of the collecting ducts become freely permeable to water. As a consequence of the high tonicity of the

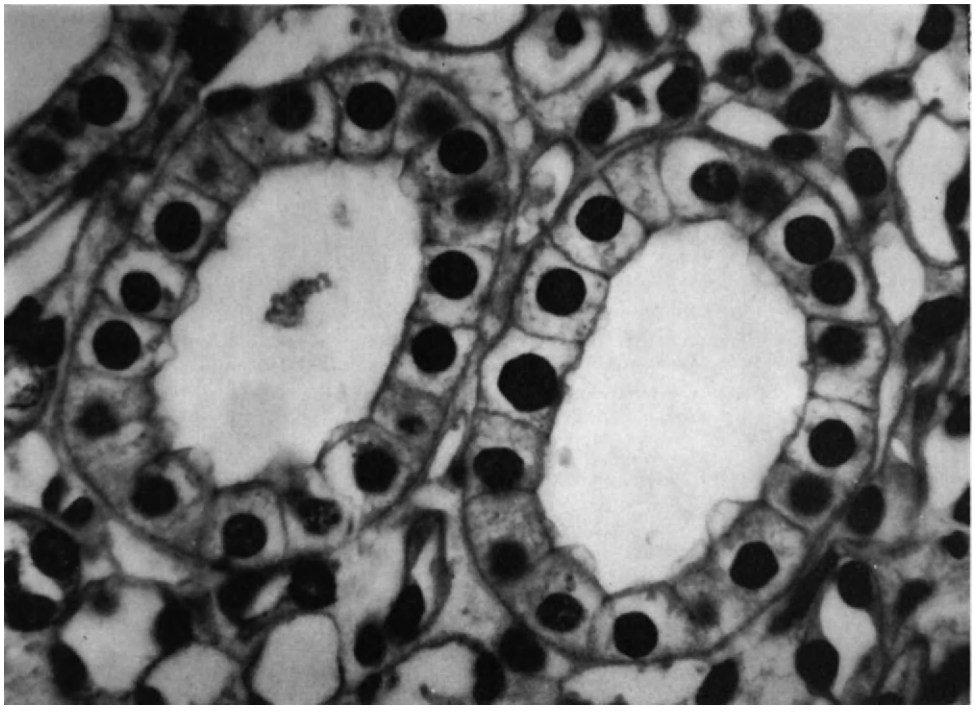

FIG. 22.15. A micrograph of two collecting ducts in transverse section. Note the marked cuboidal epithelium with clear cut cell boundaries. 8 μm.

interstitial medium in the medulla water will then leave the urine by passive transport and so the urine will become concentrated and hypertonic. By variations in the ADH content of the blood it is possible for the kidney to produce urine with a concentration which varies from 30 milliosmols to 1400 milliosmols (the concentration of the blood is normally about 300 milliosmols).

Blood Vessels (Figs. 22.17, 22.18)

The blood supply is extremely rich and the vessels are in close relation to the tubules, as would be expected from the function of the organ. The capsule has a separate blood supply.

The renal artery enters at the hilum and at once gives rise to branches (*interlobar arteries*) which pass to the boundary zone between the cortex and the medulla, where they take an arched course and are known as the *arcuate arteries*. From these arterial arches branches are given off which pass up into the cortex towards the surface: these are known as *interlobular*

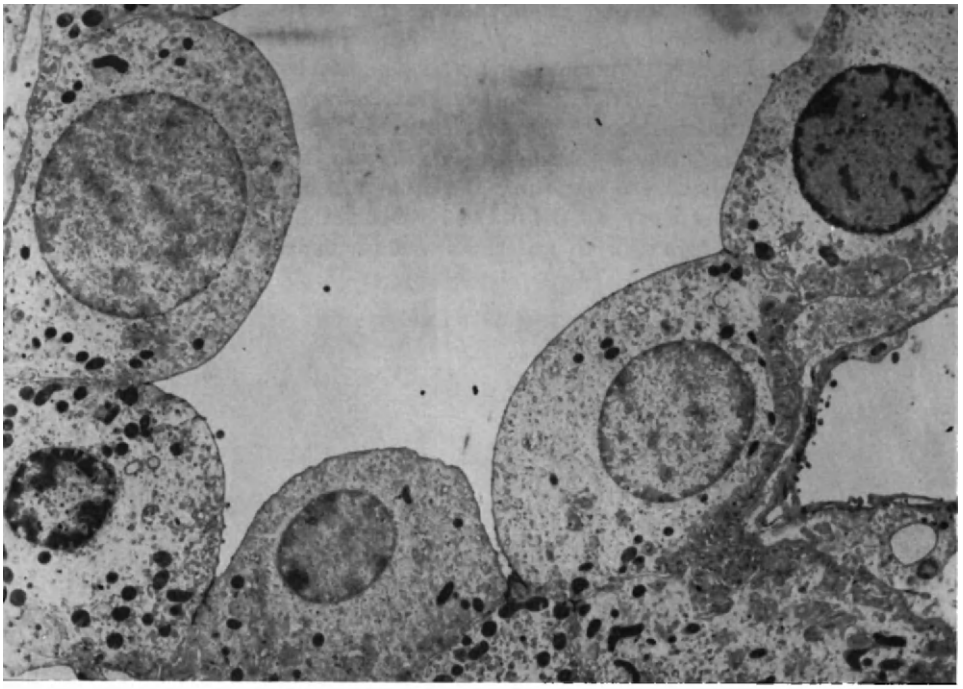

FIG. 22.16A. A low power E/M of a section through a collecting duct. 2 μm.

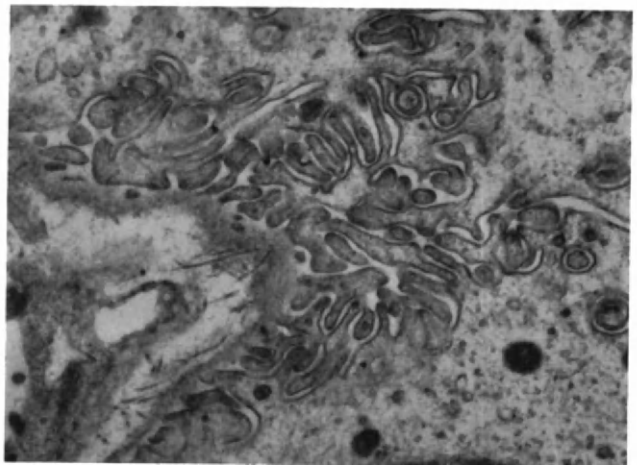

FIG. 22.16B. An enlargement of part of the basal infoldings of the cell membrane of a collecting duct. 0·5 μm.

arteries and each gives off many small branches: each branch passes to a Malpighian corpuscle as its afferent vessel and there forms the *glomerulus* (see p. 333). The efferent arterioles leaving the cortical corpuscles are considerably smaller than the afferent vessels: they then break up into networks supplying capillaries anastomosing around the convoluted tubules. These capillaries drain into *interlobular veins* which accompany the corresponding arteries, passing to the boundary zone into the venous arches and *arcuate veins*: these unite into larger veins, the blood finally leaving the kidney at the hilum in the renal vein.

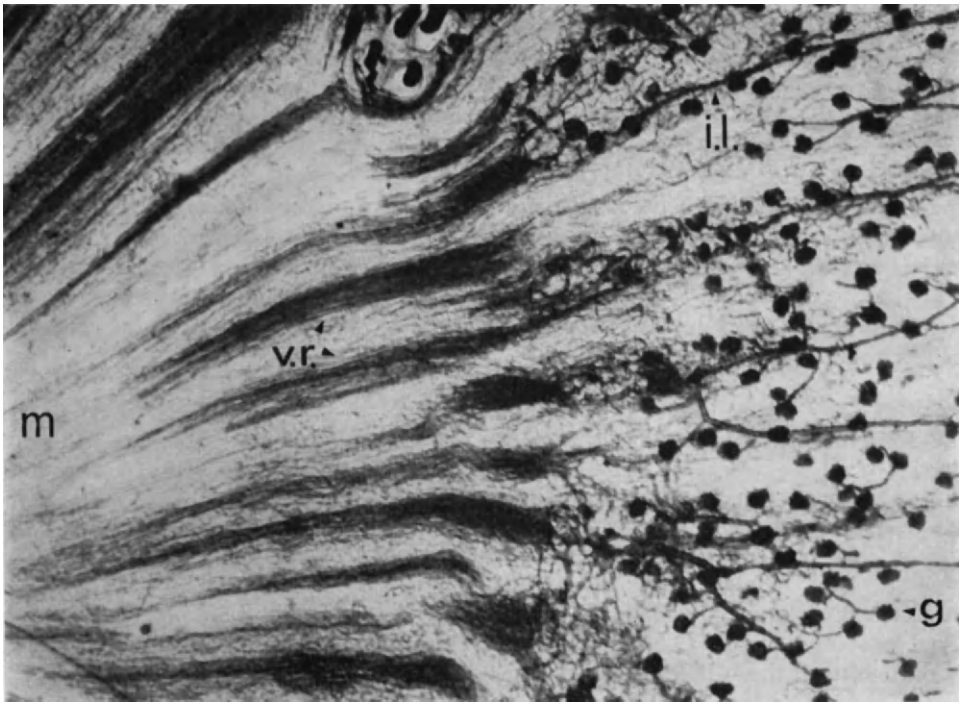

FIG. 22.17.—Part of a kidney which has had the blood vessels injected with carmine-gelatine. The cortex of the kidney is to the right of the picture. Several interlobular arteries (i.l.), and many glomeruli (g) may be seen, together with the vasa recta (v.r.) running towards the kidney medulla (m).

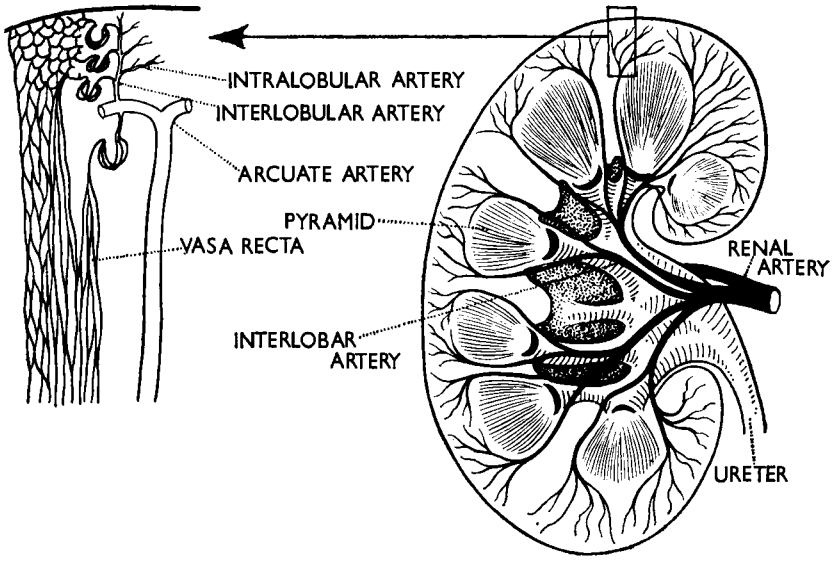

FIG. 22.18. A diagram of the organization of the blood vascular pattern in the kidney.

The efferent vessels of the glomeruli which lie in the deepest part of the cortex (juxtamedullary) differ from those in the cortex proper. Their efferent vessels are of the *same* calibre as the afferent (*cf.* above), and break up into a leash of straight vessels, the *vasa recta*, which all pass down into the medulla lying in bundles between the tubules. Their structure is that of a capillary. They form loops dipping down into the medulla and returning to the cortex and empty, typically, into a straight collecting vessel which joins the interlobular vein close to its entry into an arcuate vein.

The vasa recta because of their hairpin arrangement in close connection with the loop of Henle form an ideal countercurrent exchange system. The close and parallel arrangement of the vessels and the loop of Henle allows a near equilibrium of the osmotic calibration of the blood in the two limbs of the vasa recta and so allows the maintenance of the high osmolarity gradient from cortex to medulla which is so essential for the concentration and production of a hypertonic urine.

Lymphatic Vessels

The common, fibrous capsule of the kidney contains a lymphatic plexus, which joins the lymphatics of adjacent organs. The glandular tissue, particularly the cortex, possesses many lymphatics, the vessels making networks between the tubules and ultimately draining away in larger vessels at the hilum.

Nerves

The nerve fibres are supplied chiefly to the blood vessels. In addition, fine fibres have been described as making plexuses on the Malpighian corpuscles, and also as providing free endings between the cells of the tubules.

Atrophy, Hypertrophy and Repair

In old age and in inanition there is some atrophy of the kidney. Atrophy of a glomerulus, following interference with its blood supply, is followed by atrophy of its tubule. Blockage of the ureter, particularly intermittent obstruction, produces increased urine pressure in the kidney; this is followed by dilatation of the renal pelvis (hydronephrosis), and stretching of the kidney substance with subsequent atrophy.

The epithelium of the tubules has great powers of repair: new tubules are not formed, but the entire lining of an existing one can be renewed if damaged.

If one kidney is removed or severely damaged functional hypertrophy of the other kidney occurs, particularly in young people. There is enlargement of the existing units, but no new formation of tubules.

THE URETER (Fig. 22.19)

The ureter is continuous with the pelvis of the kidney: the structure of their walls is similar, consisting of an outer fibrous coat, a middle muscular coat and an inner lining of mucous membrane.

The *fibrous coat* consists of loose connective tissue containing many large blood vessels: it is continuous with the surrounding connective and adipose tissue.

The *muscular coat* usually consists of two layers of smooth muscle, the inner longitudinal and the outer circular: the lower third of the ureter has an additional outer layer of longitudinal muscle. The muscle fibres do not form dense, regular coats, but run in loose strands separated by connective tissue and elastic networks.

The *mucous coat* is lined by transitional epithelium: there is no clearly defined basal lamina (possibly because of the need for stretching) and the underlying connective tissue is loosely arranged and contains many capillaries. Diffuse lymphoid tissue is frequently found in the mucous coat. Owing to post-mortem contraction of the muscle the lumen of the ureter often appears star-shaped in section.

Blood Vessels and Lymphatics

The large blood vessels run in the fibrous coat: they give off branches to provide capillary networks in the muscle coat and in the mucous layer. The veins and the lymphatics follow the arteries.

Nerves

Non-myelinated fibres supply the muscle coats and the blood vessels. Myelinated fibres (probably afferent) supply the mucosa, where they lose their myelin sheath and end chiefly among the epithelial cells. Scattered groups of autonomic nerve cells are found in the muscle coat.

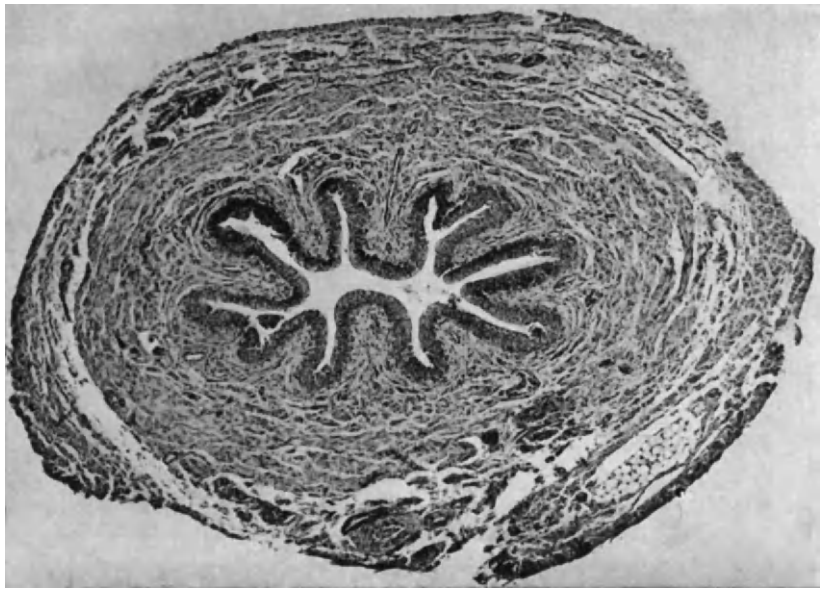

FIG. 22.19. The ureter in transverse section. Note the typical stellate lumen and the thick muscular coats. 150 μm.

THE BLADDER (Fig. 22.20)

The wall of the bladder is similar to that of the ureter. The muscle coat is thick and the three layers are not sharply marked off from one another: the middle, circular layer is the thickest, and near the urethral orifice provides a sphincter. Gland-like invaginations of the epithelium are sometimes found, particularly near the urethral opening: the cells of these glands secrete mucin. The epithelium of the bladder is transitional (p. 36 and Fig. 22.21) and so is well adapted for preventing the passage of the urinary constituents into the cells and underlying tissues. This type of epithelium is also well suited to accommodating the large scale distension which is found in the bladder.

The ureters pierce the bladder wall obliquely, so that the pressure of the bladder contents keeps the openings closed. In addition, there is a flap of bladder mucous membrane that acts like a valve, preventing backflow. Where the ureter runs obliquely through the thick bladder wall the circular muscle disappears and longitudinal muscle fibres occupy most of the mucous membrane: when these contract the ureteric orifice is opened.

The blood vessel supply of the bladder is like that of the ureter: lymphatics are said to be

present only in the muscle coats. The nerve supply is complex: many ganglia and isolated autonomic nerve cells are present in the fibrous coat and in the muscle coat. From these ganglia arise bundles of non-myelinated fibres that pursue a wavy course in the muscle coat and supply the muscle fibres. For details of the part played by the autonomic innervation of the bladder in micturition, a text book of physiology or anatomy should be consulted. A nerve plexus is present in the mucosa, associated with various types of sensory nerve endings: it also supplies varicose fibres to end among the epithelial cells. Pacinian corpuscles may occur in the fibrous coat.

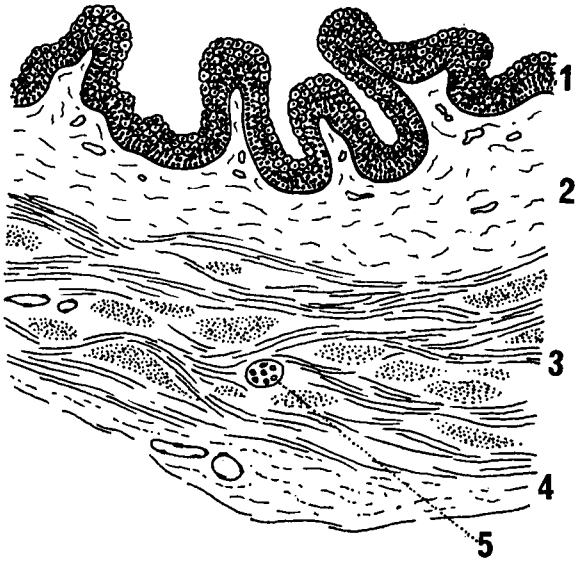

FIG. 22.20A. A diagram of the bladder wall in the relaxed condition. 1, transitional epithelium; 2, connective tissue; 3, smooth muscle; 4, fibrous layer; 5, small group of autonomic nerve cells.

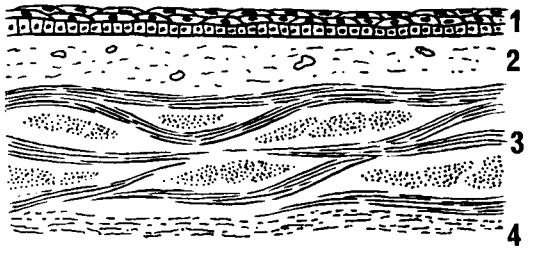

FIG. 22.20B. When the bladder is distended the rugae in the wall disappear. Numbering as in 22.20A.

Variations in the Ureter and the Bladder under Different Conditions

(1) **Degree of Physiological Stretch.** Stretching of the wall by increase of contents produces marked changes in its appearance. If the viscus is stretched the folds in the walls disappear: the epithelium becomes thinner and the cells flattened out parallel to the surface: the muscle coats are stretched so that the direction of the fibres is easily made out. If the viscus is empty and contracted the wall is thrown up into folds (Fig. 22.20): the epithelium becomes thick and the cells may even appear columnar: the loosely arranged muscle coats make thick folds and masses, the various groups are not marked off from one another and are separated by wavy strands of connective tissue.

(2) **Presence of Injurious Substances in the Urine.** Some substances eliminated by the kidney, or even produced in the urine while this is in the bladder, may have an irritant effect on the mucous lining: similarly infective agents may reach the bladder contents and irritate the lining. The epithelium is readily shed in such conditions and the underlying tissues may then be affected. This desquamation is increased if the urine is ammoniacal.

(3) **Effect of Obstruction to Outflow.** Obstruction is most often due to constriction of the urethra by enlargement of the prostate or to the presence of stones. This results in dilatation, due to accumulation of urine and difficulties in micturition. The walls of the bladder and ureters generally react by showing hypertrophy of the muscle coats. In addition, a sacculation of the bladder wall may result as the mucosa is bulged into the interstices of the feltwork of muscle.

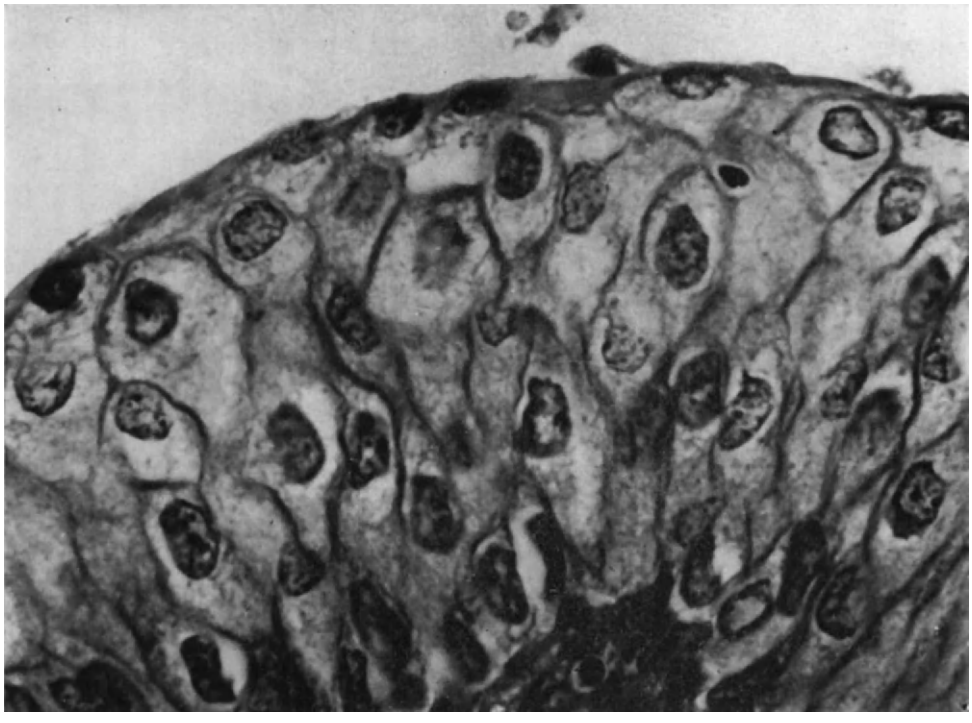

FIG. 22.21. A micrograph of the transitional epithelium of the bladder. Note the clearly defined layer of "capping" cells and the underlying intermediate and basal cells. 10 μm.

THE URETHRA

Male Urethra

The male urethra is 18–20 cm. long and is divided into three parts. The short proximal part, surrounded by the prostate gland, is the *prostatic part*: the second very short *membranous part* extends from the prostate to the bulb of the corpora cavernosa of the penis: the *cavernous* (*penile*) part extends throughout the penis. The two ejaculatory ducts and the numerous small ducts of the prostate gland open into the prostatic portion. The wall consists of three coats, the mucous coat, the submucous coat and the muscular coat.

Mucous Coat. The lining epithelium is transitional in the prostatic part, and pseudo-stratified columnar elsewhere, except in the terminal portion where it is squamous stratified. Occasional goblet cells are present. The basal lamina is well marked and beneath it is a vascular connective tissue stroma rich in elastic fibres.

The surface shows many recesses, the *lacunae (of Morgagni)*: some of these are continued into branching mucous glands, the *urethral glands (of Littré)*, lined by columnar epithelium and extending into the submucous layer: the glands are most numerous in the penile portion.

Submucous Coat (Fig. 22.22). This layer blends with the mucous coat, and consists of loose connective tissue containing scattered bundles of unstriped muscle. In the penile portion it also contains a dense network of cavernous veins, which form part of the corpus spongiosum of the urethra: this is erectile in character (see p. 366).

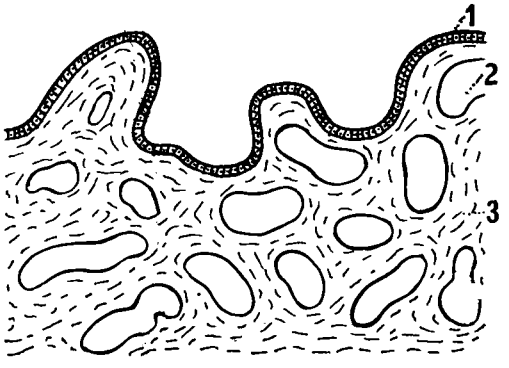

FIG. 22.22. A diagram of the arrangement of the submucous coat of the male urethra. Note the pseudostratified epithelium (1), the many cavernous veins (2) and the loose connective tissue (3).

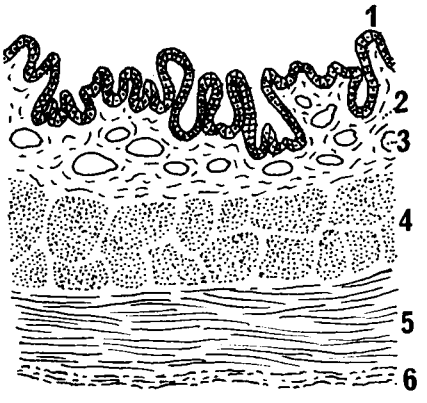

FIG. 22.23. A diagram of the organization of the wall of the female urethra in transverse section. 1, pseudostratified epithelium; 2, submucous loose connective tissue; 3, cavernous veins; 4, longitudinal muscle; 5, circular muscle; 6, areolar connective tissue.

Muscle Coat. This coat consists of inner longitudinal and outer circular layers: it is confined to the prostatic and membranous portions. The penile urethra is surrounded by a few circularly arranged muscle fibres. The membranous urethra has an external layer of striated muscle.

Female Urethra

The female urethra (Fig. 22.23) is only about 2·5–3 cm. long; it has a crescentic lumen and represents only the proximal part of the male urethra. The lining epithelium is of the transitional type near the bladder, passing over into the pseudo-stratified columnar variety and ending with a squamous stratified epithelium near the opening. Invaginations and numerous small mucous glands are present. In the lower part of the urethra there is a group of glands on each side that corresponds to the prostate in the male. The mucous coat contains a rich

plexus of cavernous veins (corpus spongiosum). The muscle coat is much thicker than in the male and consists of an inner longitudinal and outer circular layer of smooth muscle and an external sphincter of striated muscle.

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CHAPTER 23

MALE REPRODUCTIVE ORGANS

In the male the reproductive organs consist of paired testes, with their excretory ducts (epididymis, vas deferens, ejaculatory duct, urethra) and accessory glands (seminal vesicle, bulbo-urethral or Cowper's glands, prostate), and the copulatory organ (penis).

THE TESTIS (Figs. 23.1, 23.2)

The testis is a compound tubular gland enclosed in a thick fibrous capsule, the *tunica albuginea*. Outside this is the serous sac known as the *tunica vaginalis* derived from the peritoneum, necessary in order to allow for free gliding movements of the testis: the visceral layer of this sac is attached to the tunica albuginea, thus covering it with a serous epithelium, whilst the parietal layer lines the inner surface of the scrotum. Posteriorly the capsule sends into the testis a prolongation of fibrous tissue known as the *mediastinum testis*: from this radiate strong connective tissue septa that pass through the organ to fuse with the tunica albuginea, thus dividing the testis into about 250 pyramid-shaped lobules; these lobules contain the seminiferous tubules with the interstitial tissue in between them.

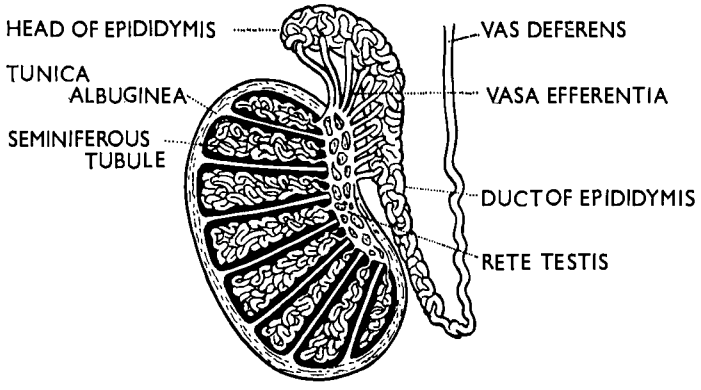

FIG. 23.1. A diagram of a longitudinal section of the testis.

The **tunica albuginea**, the **mediastinum** and the **septa** all consist of dense, regular, collagenous connective tissue containing a rich network of elastic fibres: the mediastinum contains in addition some smooth muscle. On the inner surface of the tunica albuginea the connective tissue becomes looser and very vascular (tunica vasculosa): this tissue is continuous with the interstitial tissue in between the seminiferous tubules.

The **interstitial (intertubular) tissue** fills the spaces between the seminiferous tubules, and consists of very loose connective tissue, with blood vessels and a lymphatic network, and various kinds of cells. Fibroblasts and macrophages are always present and often mast cells. In addition, there are strands and groups of specialized epithelial-like cells, the *interstitial cells (of Leydig)* (Fig. 23.4). These latter cells are large, with large spherical nuclei: the cytoplasm is eosinophil, granular, and often shows inclusions and yellowish granules. They are epithelioid cells, which form the endocrine tissue of the testis: their number and distribution vary greatly.

The **seminiferous tubules** (Figs. 23.3, 23.4) can be divided into three parts: (1) a *convoluted* part of the tubule: these begin at the periphery and occupy most of the lobules and it is here the spermatozoa are formed; (2) a *straight* part passing through the apex of the lobule to the mediastinum; the straight portions unite and form (3) the irregular network of tubules known as the *rete testis*.

(1) *Convoluted Tubule.* In any section of the testis the convoluted tubules are cut across in every direction. The wall consists of an outer connective tissue coat with its cells flattened against the tubule, a basal lamina, and a lining "stratified" epithelium which forms the germinal epithelium. In the adult two kinds of cells are present, the *Sertoli* or sustentacular cells

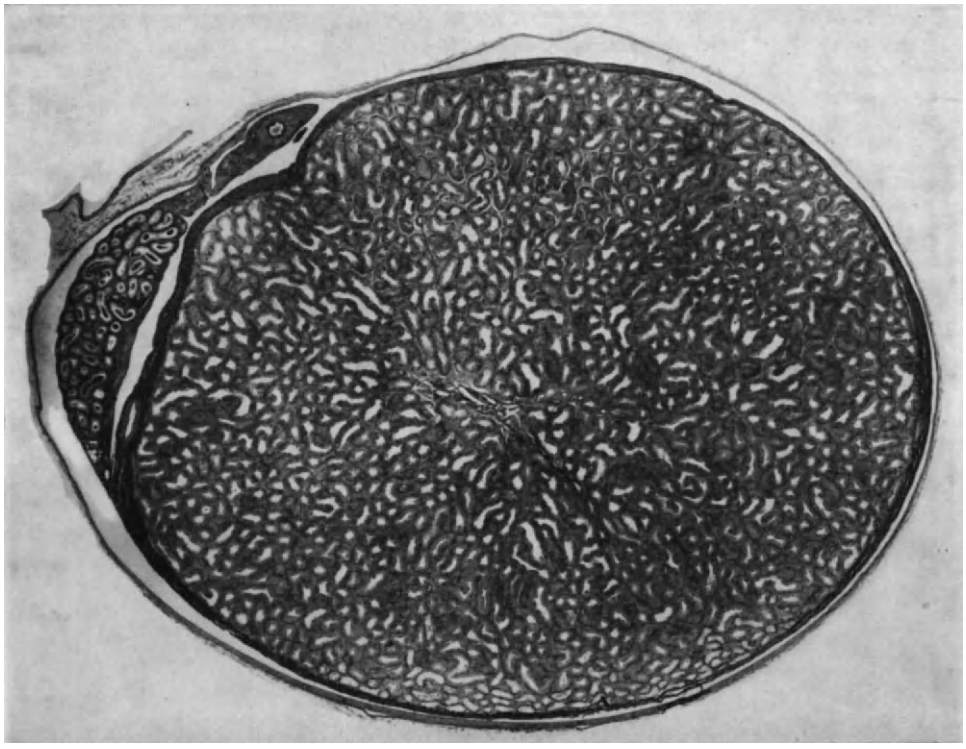

FIG. 23.2. A transverse section of the testis, showing the tunica albuginea, seminiferous tubules sectioned in various planes, and at the left, tubules of the epididymis. 2 mm.

which support and nourish the developing spermatozoa and the *germinal* cells; these latter form the vast majority of the cells in the tubules.

(*a*) **Sertoli Cells** (Sustentacular cells) (Fig. 23.5). These are slender pyramidal cells, standing at intervals on the basal lamina; crowded between them are the germ cells. The cytoplasmic outlines of the Sertoli cells cannot easily be distinguished with the optical microscope. Their nuclei are irregularly ovoid and vesicular and each contains an irregularly shaped nucleolus—this feature is a useful aid in the identification of these cells. With the E/M it can be seen that the Sertoli cell contains but little rough surfaced endoplasmic reticulum; there is, however, quite a rich accumulation of tubular smooth ER. Numerous pinocytotic vesicles may be seen at and just below the cell surface (Fig. 23.6). It has been suggested that the Sertoli cells are probably involved in the nutrition of the developing spermatids and in the secretion of fluid into the seminiferous tubule. This fluid would be useful in moving the maturing sperm along the tubule and into the vasa recta and the epididymis. The fluid, which

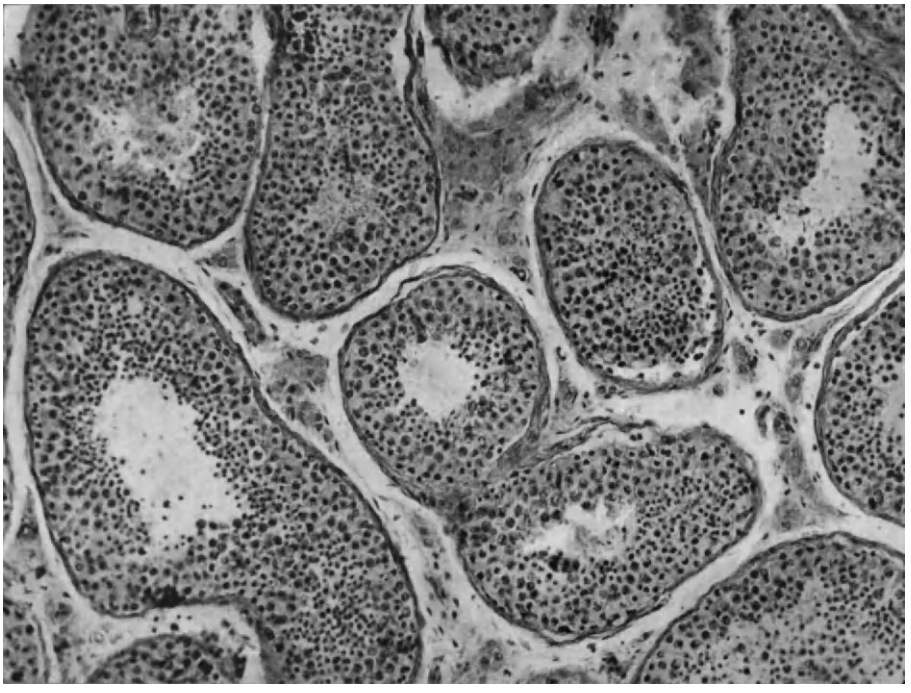

FIG. 23.3. A micrograph of seminiferous tubules of the human testis. There is some interstitial tissue visible between the tubules which contain the germ cells in active spermatogenesis.

100 μm.

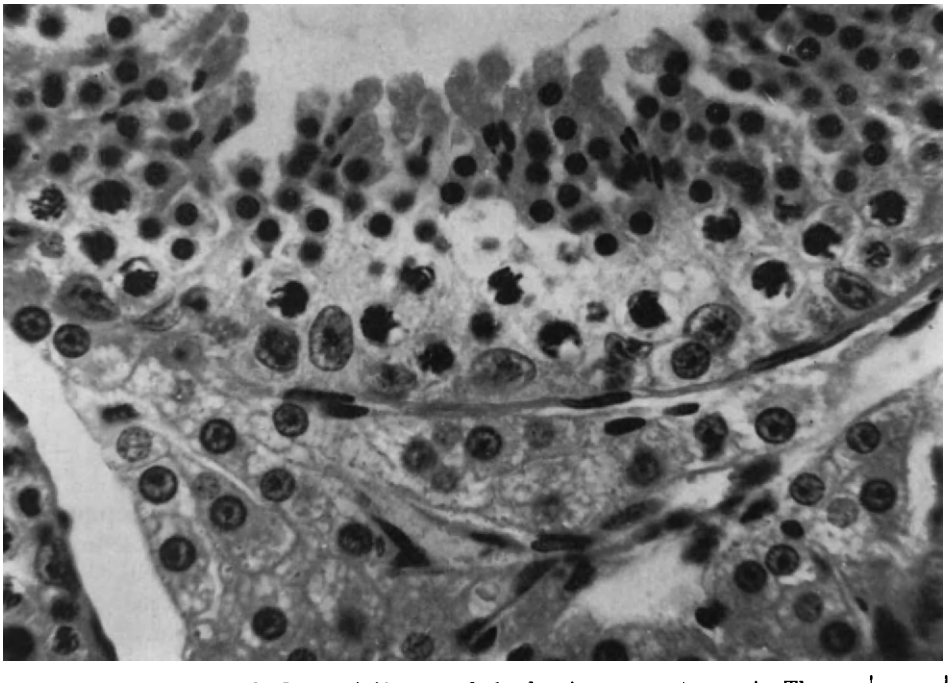

FIG. 23.4. Part of the wall of a seminiferous tubule showing spermatogenesis. The cells with small dark nuclei near the lumen at the top of the picture are spermatids. A mass of interstitial tissue showing the characteristic "foamy" cytoplasmic appearance of lipid-containing tissue after paraffin embedding is seen in the lower half of the micrograph.

15 μm.

is normally reabsorbed in the head of the epididymis, is rich in glutamic acid and inositol, together with testosterone; it also is rich in potassium ions. These findings suggest that it may also help in providing the spermatozoa with some oxidizable substrate. The testosterone may be important in stimulating activity of the cells of the epididymis, as they probably exposed to much higher concentrations of this hormone from this fluid in the lumen than reaches them from the blood. The E/M shows clearly that the spermatids invaginate deeply into the cytoplasm of the Sertoli cell but no actual cytoplasmic continuity between the cells ever seems to exist.

(*b*) **Germ Cells.** These cells in various stages of development lie between the Sertoli cells, occupying five or six layers between the basal lamina and the lumen of the tubule. Next to the basal lamina lie the *spermatogonia*: these have clear cytoplasm with their nuclear chromatin

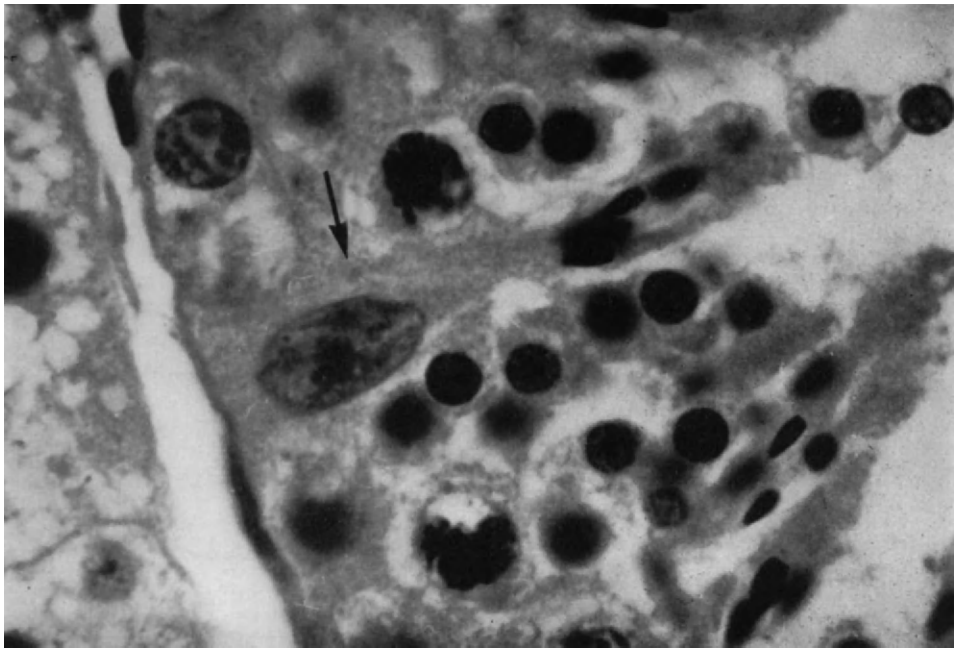

FIG. 23.5. A high power photograph of the germinal epithelium. The arrow indicates a Sertoli cell which has spermatids associated with its luminal surface; note the irregular nucleolus which is characteristic of this type of cell. 5 μm.

usually appearing as a network: in some tubules however active division of these cells is occurring. Next to the spermatogonia are the *spermatocytes*, their nuclei frequently showing active division: the outer layer of primary spermatocytes, formed from the spermatogonia, are large and spherical with large nuclei; the inner layer, formed from these by the special process of reduction division, are the much smaller secondary spermatocytes. Next to these, and formed by their division, are the *spermatids*, appearing as several layers of small round cells with round nuclei. In some places these spermatids may be seen developing into the *spermatozoa*, a process termed *spermiogenesis*. In so doing they first develop short tails and then elongate, the nucleus being situated at the head end, remote from the tail. At the same time a body known as the acrosomal granule develops in association with the Golgi material on that side of the nucleus opposite to the developing tail. From it is derived the *acrosomal cap* which, in the mature sperm, is a thin membrane covering the anterior part of the head or nuclear region. This structure is concerned in facilitating the entry of the sperm into the egg at fertilization. The cells collect in groups, with their heads pushing between the other cells to make contact with a Sertoli

cell while their tails project into the lumen of the tubule. When mature the spermatozoa lose connection with the Sertoli cells and are found free in the lumen. Spermatogenesis appears to proceed in an orderly fashion, beginning with the mitotic division of the spermatogonia. This results, in any one cycle, in the formation of two spermatogonial types which, in the rat, have been designated type-A and type-B. The latter further divide, in the manner previously described, to give rise ultimately to spermatids and, through their transformation, to spermatozoa. The type-A, however, remain dormant until type-B have divided into primary spermatocytes

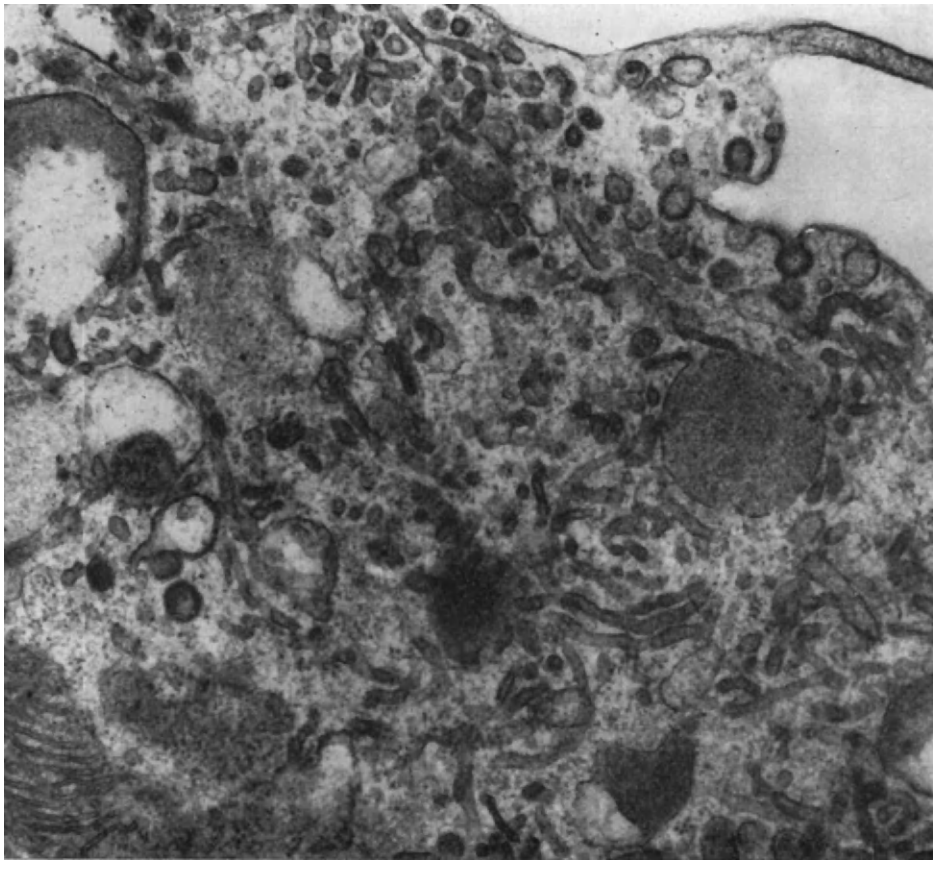

FIG. 23.6. An E/M of part of the cytoplasm of a Sertoli cell. Note the rich accumulation of tubular elements of the smooth surfaced endoplasmic reticulum at the apex of the cell and the numerous pinocytotic vesicles at the cell surface. The lumen of the seminiferous tubule is at the upper right of the micrograph. 0·5 μm.

and have completed the first meiotic division (i.e. have divided to give secondary spermatocytes) At this stage, the dormant A-type initiate a new wave of spermatogenesis, by dividing to give a proportion of new type-B spermatogonia and a proportion of type-A spermatogonia, the latter again remaining dormant until the completion of the following first meiotic division. The ripe spermatozoon (Fig. 23.7) consists of a flattened head containing the nucleus and acrosomal cap, a short cylindrical *middle piece* which harbours the *mitochondrial sheath*, and the long *principal piece*. The principal piece (often loosely called the "tail") has the same basic ultra-structure as a cilium or flagellum; there is the central *axial filament* complex but the outer fibres of the nine doublets in the centre show marked assymmetry, Nos. 1, 5 and 6 being

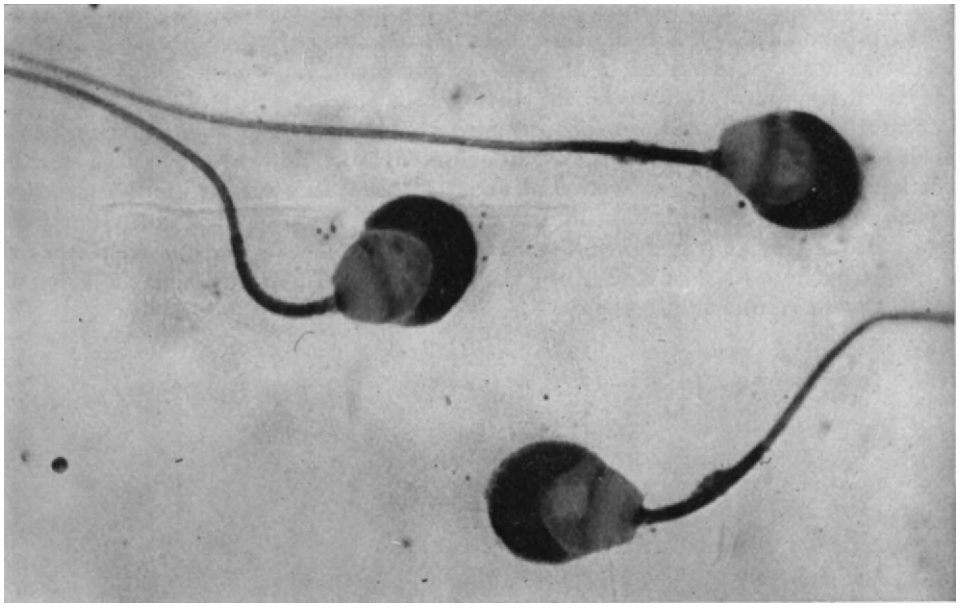

FIG. 23.7. Spermatozoa of the guinea pig. Note the prominent acrosome (staining very darkly), the head, midpiece and the principal piece (in part). 10 μm.

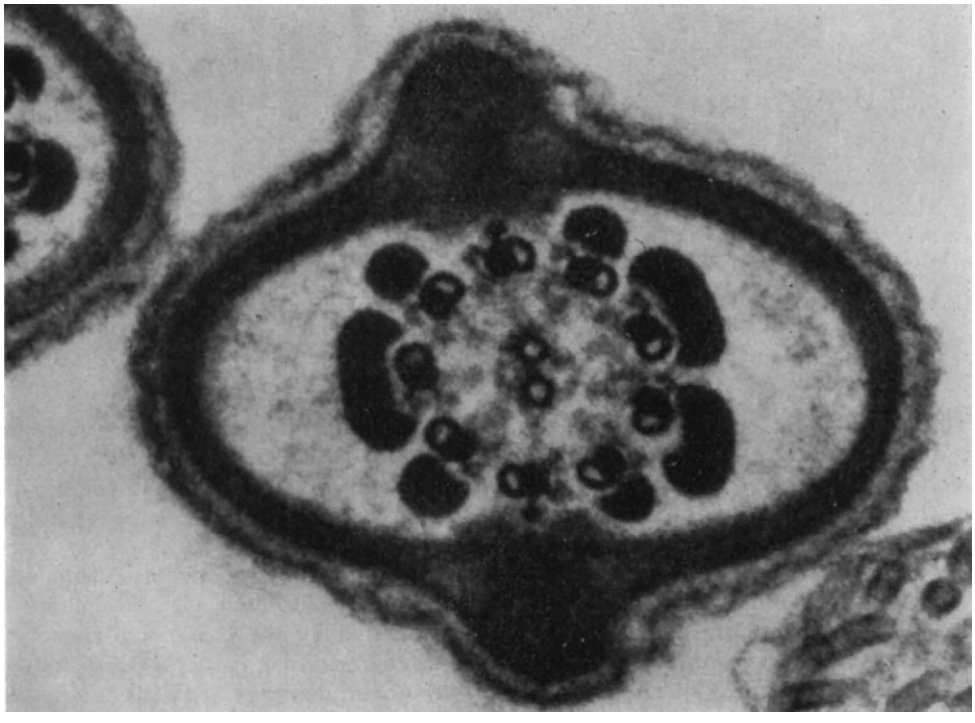

FIG. 23.8. An E/M of a transverse section through the principal piece of a mammalian spermatozoon. The axial filament complex is visible, together with the outer fibrous sheath with its two longitudinal thickenings. 0·1 μm.

larger than the others (Fig. 23.8). There is also a prominent longitudinal fibrous sheath with two ribs arranged outside the axial filament complex. Behnke has recently shown that actin-like filaments may be identified in the sperm principal piece, and he suggests that actin-myosin interactions form the basis for the sperm motility.

During the maturation of a crop of spermatozoa another series of spermatocytes has begun to develop, the cycle continuing without interruption; the total time required in man for the maturation of a sperm being estimated at about 64 days. In a section of an active testis all these phases may be seen in various tubules. In man each section through a tubule may contain three or more of the six stages of different cell associations into which spermatogenesis has been arbitrarily divided. For a detailed account of these, Clermont's paper listed in the references should be consulted.

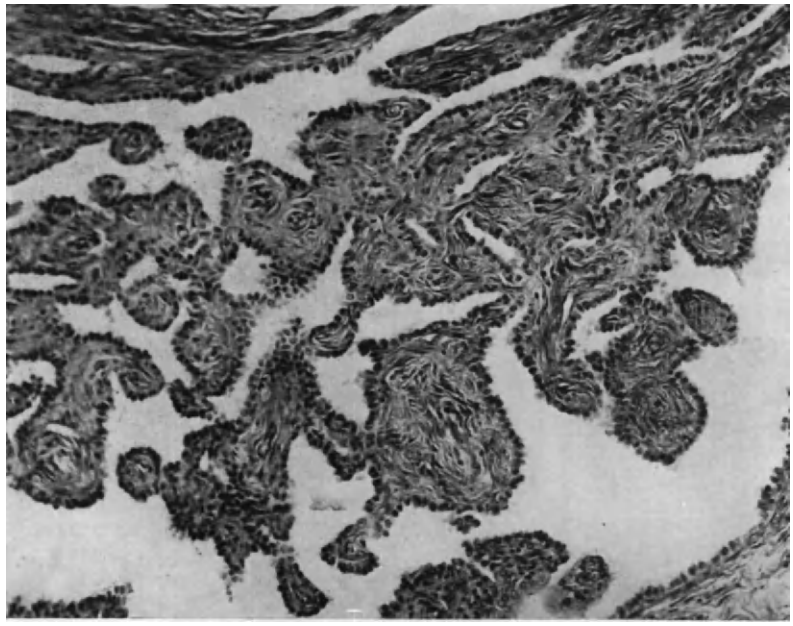

FIG. 23.9. A section through the rete testis of a rat. The large irregular anastomotic canals may be seen. 50 μm.

The somatic number of chromosomes in the human is 46. When the spermatogonia divide there are 22 ordinary pairs and one XY pair, all of which split longitudinally. At the reduction division of primary spermatocytes the XY chromosome separates so that the secondary spermatocytes are of 2 kinds, each having 22 ordinary chromosomes (*or autosomes*) and either an X or a Y sex chromosome.

(2) *Straight Tubules.* The spermatogenic tissue is limited to the convoluted tubules. The straight tubules are lined by a clear cubical or flattened epithelium with no definite basal lamina visible in the optical microscope, but surrounded by the connective tissue of the mediastinum. Here they form a system of irregular, anastomosing spaces, the *rete testis.*

(3) *Rete Testis* (Fig. 23.9). This consists of a series of large, irregular anastomosing canals, lined by low cubical or flattened epithelial cells. The 8 to 15 *vasa efferentia*, passing from the rete testis to the epididymis, are lined by columnar ciliated cells interspersed with clear glandular cells: there is a basal lamina and a thin layer of circular smooth muscle.

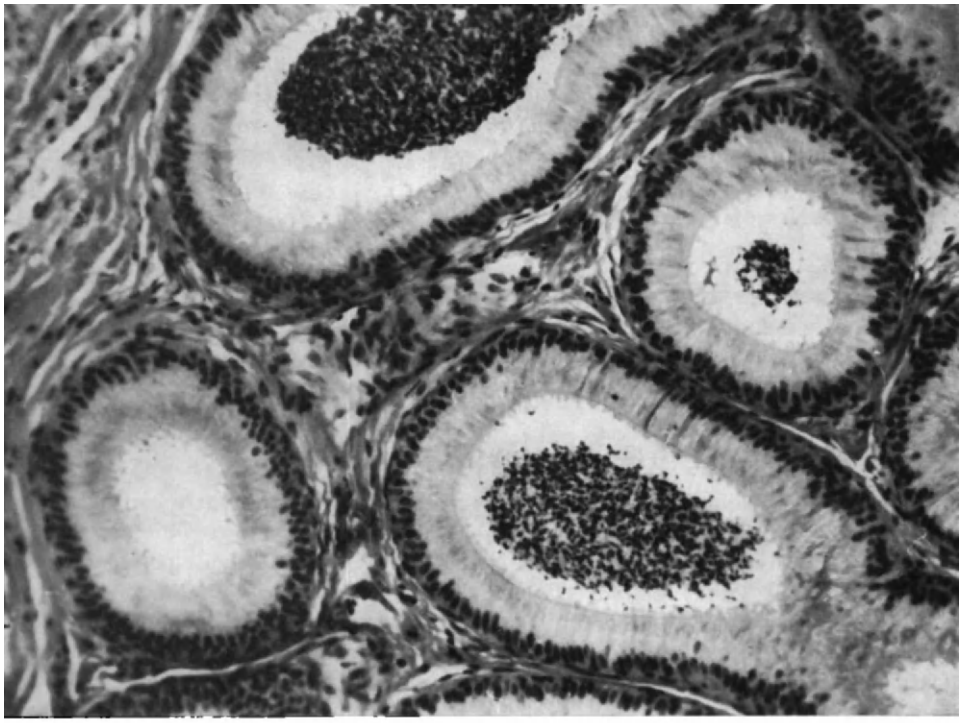

FIG. 23.10. Section through the tubules of the epididymis. The tall epithelial cells of the tubules are surrounded by a thin layer of smooth muscle and connective tissue.

25 μm.

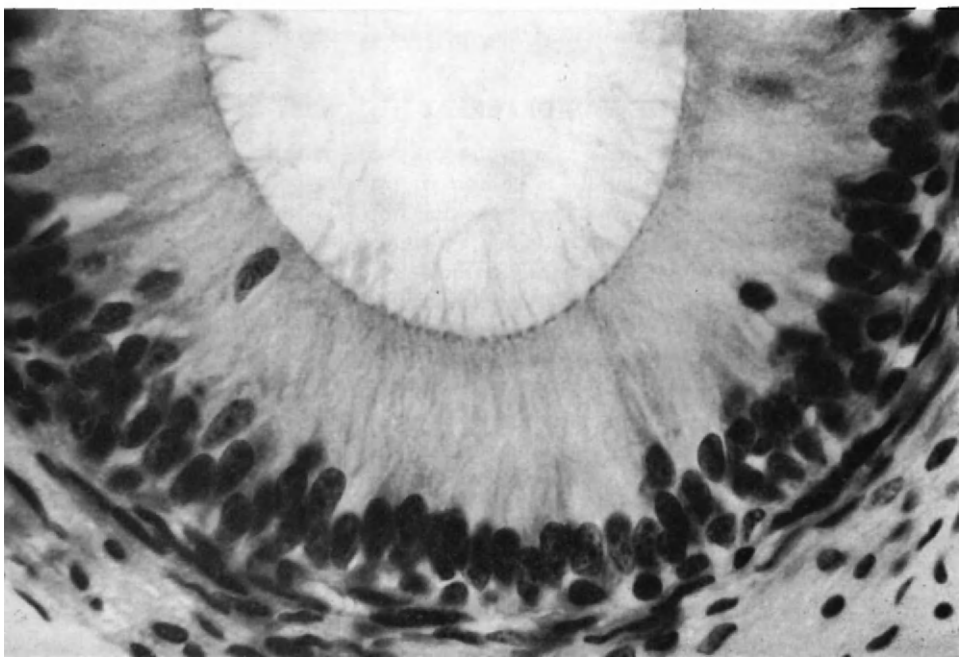

FIG. 23.11. A high power micrograph of the epithelial cells of the epididymis. The stereocilia on the luminal surfaces of the cells may be seen.

6 μm.

Blood Vessels and Lymphatics

The blood vessels ramify in the mediastinum and send branches into the septa: these provide a capillary network among the tubules and the issuing veins follow the arteries.

The interstitial tissue contains networks of lymph capillaries: similar networks are present in the tunica albuginea. These all drain to the lymphatic vessels of the mediastinum and spermatic cord.

Nerves

The blood vessels are supplied with nerve plexuses. Some observers have described similar plexuses round the seminiferous tubules. In addition, there is a very close relation between non-myelinated nerve fibres and certain cells similar in appearance to interstitial cells of Leydig that are paraganglionic in nature (see p. 222).

THE EPIDIDYMIS (Fig. 23.10)

The vasa efferentia converge and ultimately form the single, greatly coiled tube of the epididymis; it is estimated to have a length of between 4–6 metres in the human. Its lumen is lined by tall columnar cells with elongated nuclei (Fig. 23.11). Small polyhedral cells lie between the tall cells. The columnar cells bear bunches of non-motile filaments or *stereocilia* and contain granules, lipid droplets and vacuoles. There is evidence that the droplets may pass out of the cells into the lumen and provide the basis of the secretion which will form the bulk of the semen in which the spermatozoa are suspended. The cells of the epididymis are characterized at the E/M level by the presence of an extensive network of tubules of the smooth-surfaced endoplasmic reticulum and by a very large supra-nuclear Golgi apparatus. Outside the basal lamina of the tubule of the epididymis is a very rich capillary network and a thin layer of smooth muscle arranged circularly around the tubule; this latter may be of importance in propelling the sperm along the tubule, although undoubtedly the secretion of fluid in the seminiferous tubules mentioned on p. 355 also plays a part in this.

THE VAS DEFERENS (Fig. 23.12)

As the duct of the epididymis passes into the vas deferens the wall becomes much thicker (see Fig. 23.12). The lining epithelium is columnar, pseudo-stratified and usually possesses stereocilia; outside the basal lamina is a vascular fibro-elastic layer of connective tissue. Outside this again is the muscle coat: this is very thick and usually three layers can be distinguished, a thin inner longitudinal coat, a thick middle circular coat, and a thick outer longitudinal coat. At the periphery is a layer of fibrous connective tissue.

Near its ending the vas deferens dilates into the *ampulla*. Here the wall has the same structure, but branching tubular glands are present. The duct then continues as the short, straight, ejaculatory duct, pierces the prostate gland, and opens into the prostatic part of the urethra. The ejaculatory ducts are lined by a columnar epithelium (often containing a large quantity of yellowish pigment granules) which becomes transitional in type near their opening. The muscle coats are thinner than in the vas deferens and tend to merge with the muscle of the prostate.

The structure of the urethra has been described above (see p. 351).

Blood Vessels and Lymphatics

The ducts are all provided with a rich vascular capillary network beneath the epithelium. The lymphatic capillaries drain into the larger vessels of the spermatic cord.

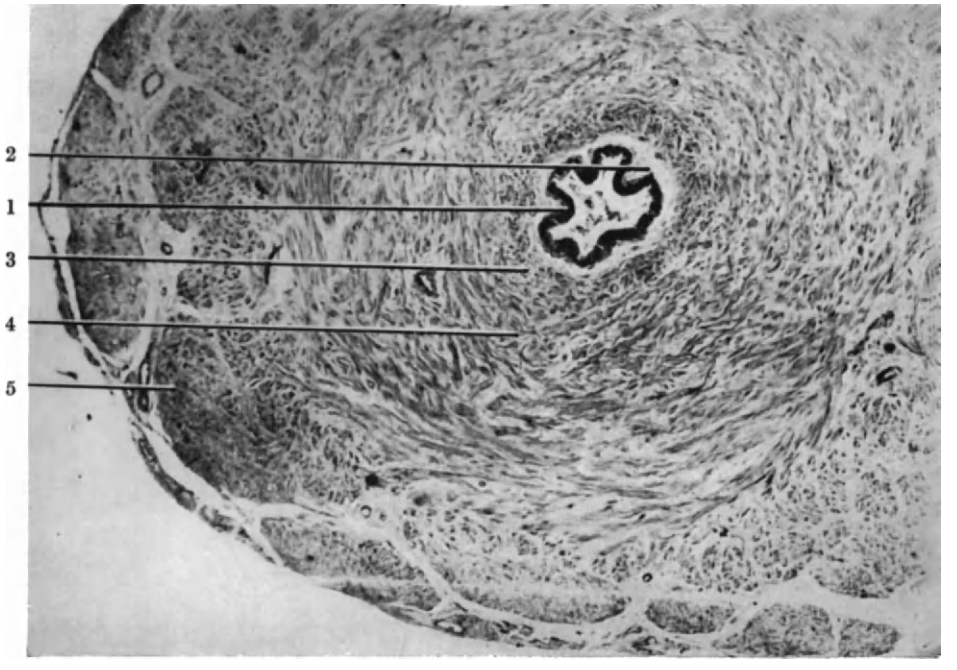

FIG. 23.12A. A section through the vas deferens of the monkey. 1, columnar pseudostratified epithelium; 2, connective tissue; 3, internal layer of longitudinal muscle; 4, circular muscle; 5, external longitudinal muscle.

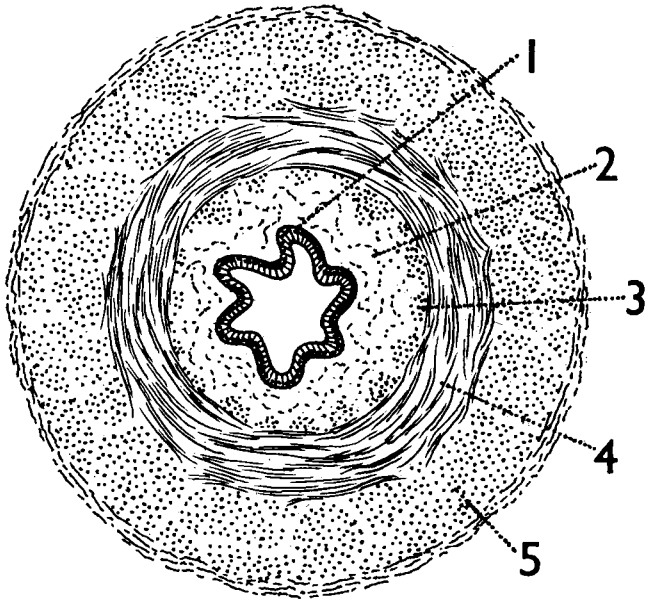

FIG. 23.12B. A diagram of a transverse section through the vas deferens. The numbering is as in Fig. 23.12A.

Nerves

The walls of the ducts contain plexuses of non-myelinated fibres which supply the smooth muscle and the mucosa. Small autonomic ganglia are present in the epididymis.

The *spermatic cord* runs from the testis in the scrotum through the inguinal canal. It carries the vas deferens, numerous arteries, a plexus of convoluted veins of the pampiniform plexus with thick muscular walls, nerves, lymphatics, longitudinal strands of striated muscle (cremaster) and connective tissue.

ACCESSORY GLANDS

(1) **Seminal Vesicle** (Fig. 23.13). This is an evagination of the vas deferens for the elaboration of a thick secretion which forms one of the components of the semen; it has the same general structure as the vas. It is an elongated and convoluted tubular, glandular sac, the

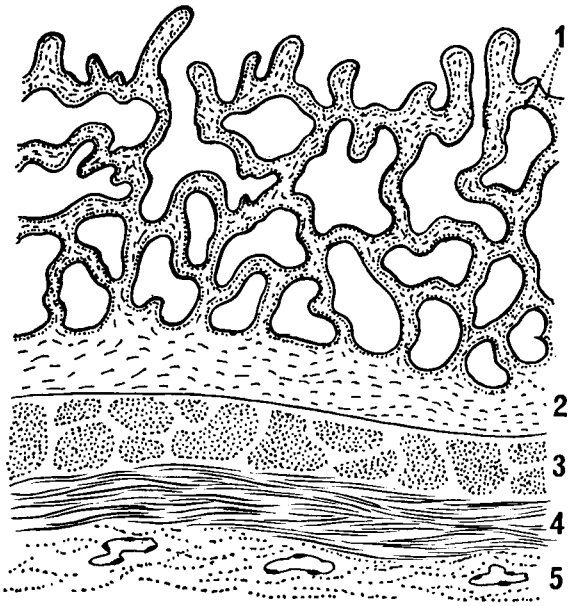

FIG. 23.13. A diagram of the organization of a vertical section through the wall of the seminal vesicle. 1, epithelium lining the organ and its foldings; 2, underlying loose connective tissue; 3, circular muscle; 4, longitudinal muscle; 5, fibrous coat.

convolutions held together by vascular connective tissue provided with elastic networks. The lining of the tubule consists of superficial columnar cells and basal round cells. The cells contain much yellow, fatty pigment and secretion granules: on their free surface drops are often visible, being shed into the lumen. Beneath the basal lamina is a layer of vascular connective tissue, and outside this a layer of circular smooth muscle. The mucous membrane is thrown up into many thin folds that branch and anastomose (see Fig. 23.13).

(2) **Prostate Gland** (Fig. 23.14). The prostate gland, composed of 30–50 small tubulo-alveolar glands, surrounds the beginning of the urethra and opens into its floor by about twenty ducts; it is pierced by the ejaculatory ducts. The glandular tissue consists of branching tubules with dilated endings that appear in a section as large alveoli with folded walls; they are lined by a non-ciliated columnar or cubical secretory epithelium supported by small basal cells. The secretory cells contain droplets in the cytoplasm between the nucleus and the alveolar lumen, and these are discharged during the secretory cycle. The cells are also rich in

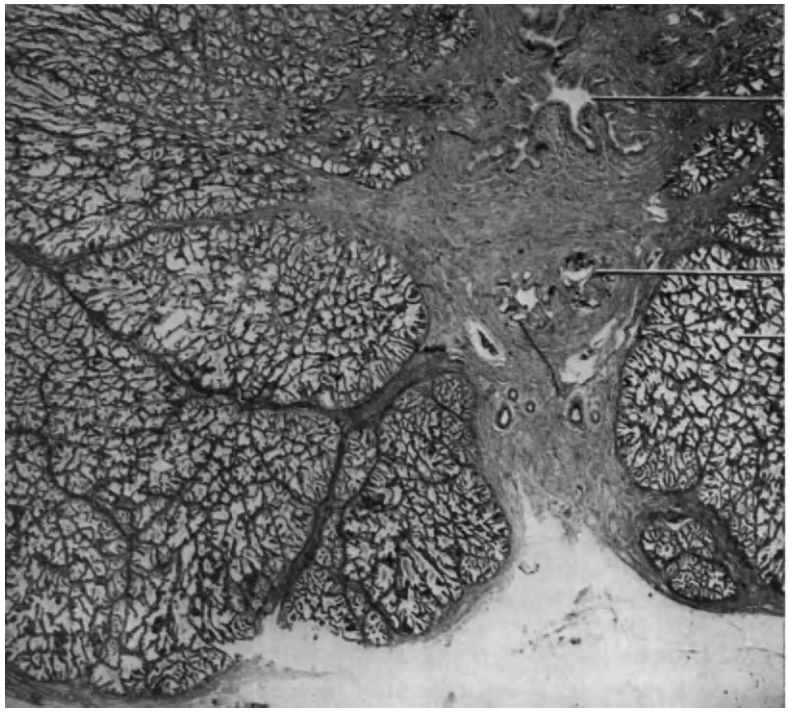

FIG. 23.14. A section of the prostate, showing part of the urethra. 1, urethral lumen; 2, ejaculatory duct posterior to the urethra; 3, glandular tissue of the prostate.

1 mm.

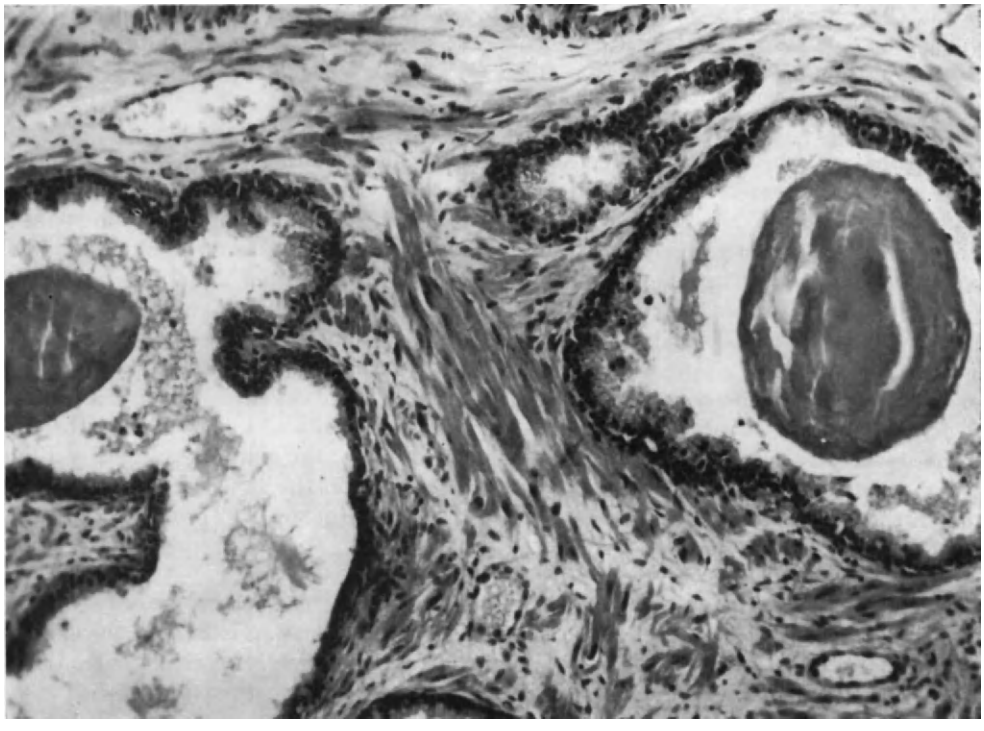

FIG. 23.15. A high power micrograph of the alveoli of the prostate containing corpora amylacea. Note the strands of smooth muscle in the connective tissue between the alveoli.

35 μm.

acid phosphatases. They are very sensitive to the concentration of androgens, becoming very much reduced in size and activity after castration—an effect which can be reversed by the injection of male sex hormone. The alveoli often contain colloidal masses of secretion termed *corpora amylacea* (Fig. 23.15). There is no definite basal lamina, but a layer of very vascular and elastic connective tissue surrounds the gland to form a capsule which sends broad radiating trabeculae into its substance. The interalveolar tissue consists of dense connective tissue, with collagen and elastic networks, and of numerous strands of smooth muscle. Round the urethra the muscle forms a thick ring.

Between the ejaculatory ducts is the *prostatic utricle*, a foetal remnant of the paramesonephric duct, consisting of a blind sac that opens into the prostatic urethra lined by ciliated epithelium that dips down to form short glands. The prostate secretion is a thin opalescent liquid with a pH of about 6·5; it is rich in amylase and proteases, in particular in fibrinolysin. It is from the prostate that the semen receives its high concentration of citric acid and of acid phosphatase. Recently the prostate and seminal vesicles have been shown to be a rich source of substances known as *prostaglandins*: these are unsaturated hydroxy acids which, amongst other functions, stimulate the smooth muscle of the female genital tract to contract.

(3) **Bulbo-urethral Glands** (Cowper's glands). These are two compound racemose glands opening into the cavernous part of the urethra. The secreting portions are often dilated into alveoli: these are lined by columnar or cubical cells, secreting a substance like mucus and rich in sialo-proteins. The interstitial connective tissue contains smooth muscle.

Blood Vessels and Lymphatics

The blood vessels supplying the glands ramify in the capsule and trabeculae, providing capillary networks for the alveoli. The veins and lymphatics accompany the arteries.

Nerves

The smooth muscle is supplied by non-myelinated fibres. In addition, in the prostate are found small autonomic ganglia, and also in the interstitial tissue various types of sensory nerve endings belonging to myelinated fibres. Free nerve endings have been described among the secreting cells.

THE PENIS (Fig. 23.16)

The penis consists chiefly of erectile tissue running longitudinally in three cylindrical masses, the two *corpora cavernosa* lying dorsally side by side, and the median ventral *corpus spongiosum* surrounding the urethra. All the structures are enclosed in a common connective tissue capsule rich in elastic fibres and attached rather loosely to the overlying skin. In addition, each has its own fibrous capsule, or tunica albuginea, which contains dense layers of collagen fibres and elastic networks: the fibrous partition between the corpora cavernosa is deficient near the tip of the penis. The capsule of the corpus spongiosum is relatively thin, and contains many elastic fibres, thus allowing of free expansion. The corpus spongiosum ends in a mushroom-shaped enlargement covering the ends of the corpora cavernosa, and known as the *glans penis*. The skin covering the penis is thin, while the subcutaneous layer is thick and contains smooth muscle and large numbers of nerve bundles: the glans is covered by a fold of skin, the *prepuce*. Many sensory nerve endings are present in the subcutaneous tissue, particularly in the glans. In some animals, bone and fibro-cartilage are also present.

Blood Vessels and Erectile Tissue (Fig. 23.17)

Erectile tissue consists of a sponge-like mass of wide, irregular venous spaces between the ends of the arteries and the beginnings of the veins. When the organ is relaxed the spaces are collapsed and nearly empty: in erection they are filled with blood under pressure, this producing the enlargement and rigidity of the penis. These cavernous spaces are lined with vascular

endothelium; between them lie the trabeculae, which are in reality the fused and modified walls of the original veins; they consist of connective tissue with thick collagen bundles and elastic networks, and also smooth muscle fibres. The spaces in the corpora cavernosa are largest in the central zone and diminish in size toward the periphery: in the corpus spongiosum the lacunae are all of uniform size. The internal pudendal arteries supply branches to the dorsum of the penis (dorsal artery), branches to the corpora cavernosa (deep arteries), and

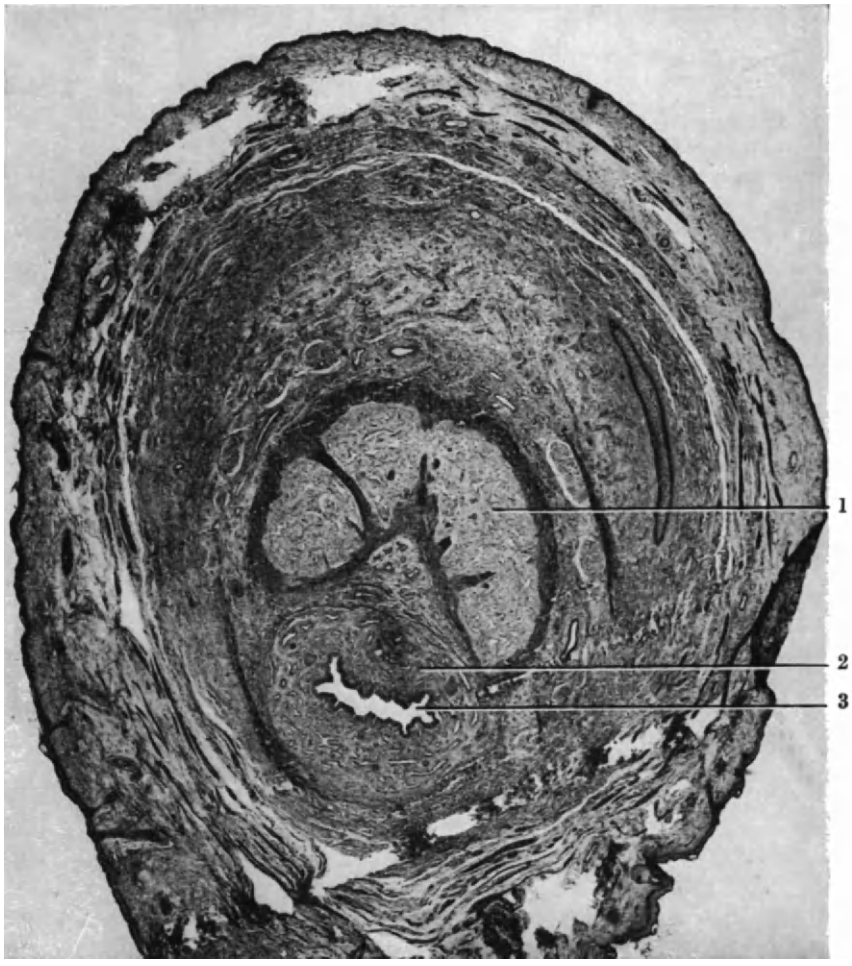

FIG. 23.16. A transverse section of the penis of a full term human foetus. 1, corpus cavernosum; 2, corpus spongiosum; 3, urethra.

branches to the corpus spongiosum (artery to the bulb). The dorsal artery supplies the albuginea and the larger cavernosa trabeculae, and the blood drains into the plexus of albugineal veins: when the organ is flaccid most of the blood passes this way. The deep arteries are particularly concerned with erection: they run lengthwise in the trabeculae through the corpora cavernosa opening directly into the cavernous spaces. In the collapsed condition of the penis many take a looped course—forming the so-called helicine arteries: during erection these are straightened out. Many of these arteries have a very thick tunica media of circular muscle, and often have localized longitudinal thickenings of the intima: these fill up the lumen when the

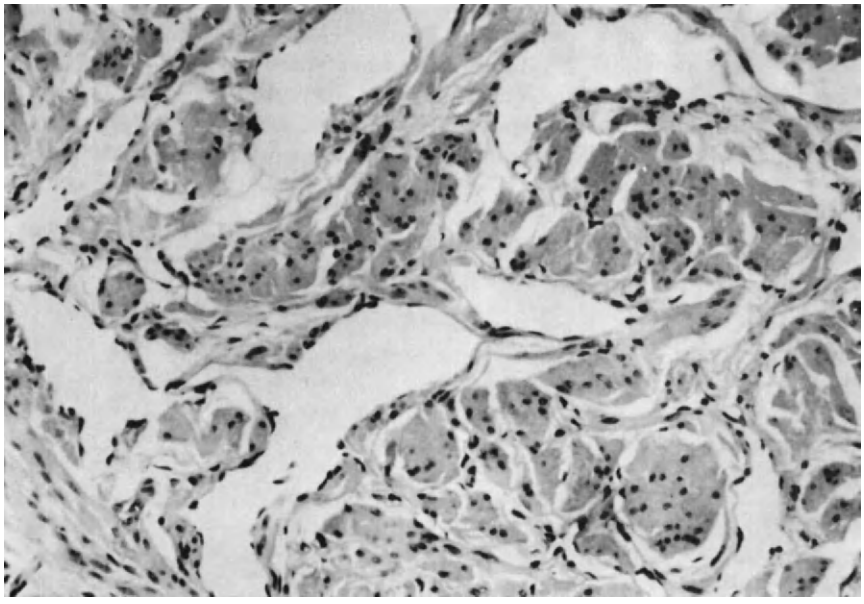

FIG. 23.17A. A low power micrograph of erectile tissue showing the cavernous spaces and trabeculae. 60 μm.

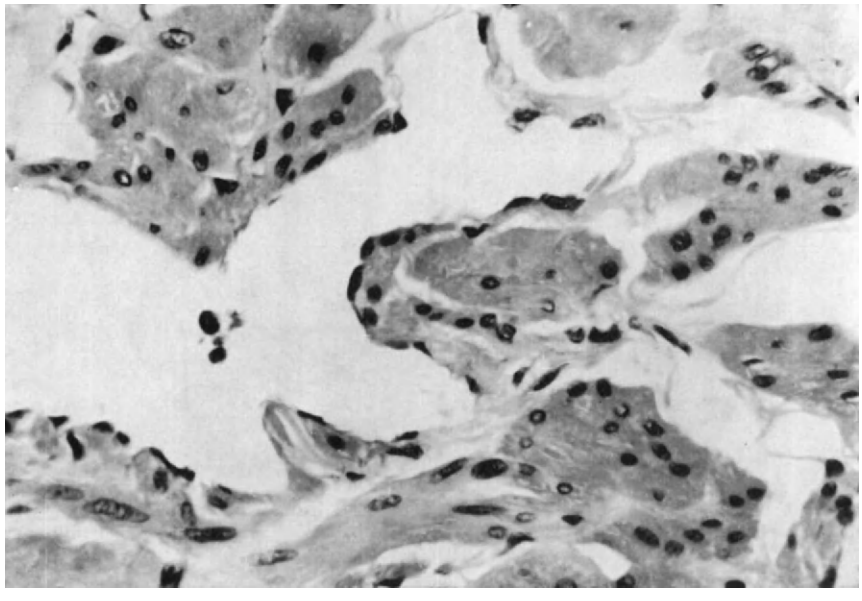

FIG. 23.17B. High power view of erectile tissue. Note the vascular spaces are lined by endothelium; many collagenous trabeculae occur between these cavernous spaces. 40 μm.

muscle contracts, and so stop the blood supply. The arteries are actively concerned in the mechanics of erection. All the smooth muscle in the arteries and erectile tissue relaxes, and the cavernous spaces are suddenly filled with blood from the helicine arteries: the filled central large spaces tend to compress the smaller peripheral ones and the thin-walled veins under the

albuginea into which they drain. Consequently the outflow of blood is much diminished and the whole organ becomes tense. At the end of erection the helicine arteries contract and the blood inflow is cut off: the muscle in the trabeculae regains its tone and the stretched elastic fibres recoil: the blood is thus slowly forced out into the venous plexus and drained away.

The corpus spongiosum swells during erection due to the increased blood supply, but the albuginea is very elastic, the cavernous spaces are of uniform size and the outflow of blood is not greatly interfered with: the spongiosum does not therefore become rigid.

Lymphatics

Dense lymphatic networks are found in the skin, with deeper networks in the glans.

Nerves

Many sensory endings are present: there are free endings in the epithelium of the glans, the prepuce and urethra, and in the sub-epithelial cutaneous tissue; in the papillae of the skin and subcutaneous tissue are found end bulbs and Pacinian corpuscles. The smooth muscles are all supplied with non-myelinated nerve fibres.

THE SEMEN

Some secretion is added to the forward moving mass of spermatozoa from the cells of the vasa efferentia. The sperms may remain in the duct of the epididymis for months, and are here mixed with the thick nutritive secretion of its cells. The sperms then pass quickly through the vas deferens, and the mass receives the secretion from the seminal vesicles during ejaculation. At this time is also added the liquid prostatic secretion forced out by the contraction of its smooth muscle. In the urethra, the bulbo-urethral glands add their secretion also.

The semen is the liquid finally ejaculated from the urethra, and thus consists of a mixture of the spermatozoa derived from the testis together with the various secretions. Typically semen would contain about 225 mg. fructose, 375 mg. citric acid and small amounts of lactic, pyruvic acids, inositol and sorbitol in 100 ml. Traces of ascorbic acid and zinc would also be present. There is, however, a high concentration (70 mg/100 ml.) of glyceryl phosphoryl choline and a K/Na^+ ratio of 1 : 2. It should be remembered that the spermatozoa are only in contact with the other seminal constituents for a few minutes at the most, as mixing only begins at the time of ejaculation. The function of the many components of the semen is not yet entirely clear.

In the semen degenerating cells desquamated from the walls of the ducts and corpora amylacea are usually present, together with immature spermatozoa. Fertile human semen may contain up to 20 per cent of abnormal sperms and a few polymorphonuclear leucocytes. Abnormal sperms may show double heads, kinked or double tails, or retention of protoplasm in the head: sometimes the staining reaction is changed: such forms abound in non-fertile semen.

Variations in the Male Reproductive Organs under Different Conditions

(1) Age

None of the sexual organs are completely developed before puberty and many tend to degenerate in senility.

Testis. The seminiferous tubules are developed from the medullary (or sex) cords derived from the foetal germinal epithelium. Up to about six foetal weeks there is an "indifferent" period during which development follows the same course in both sexes: after this, differentiation is quite clear, and development occurs rapidly in the male. Before puberty the cells of the seminiferous tubules are all more or less the same, there is no clear lumen in the seminiferous tubules until about 3–4 years of age: there is much intertubular tissue containing large

interstitial cells. At puberty the germinal cells proliferate and begin to give rise to spermatozoa, the first few generations being usually abortive: the Sertoli cells differentiate at this time. Spermatogenesis continues throughout life; in senility, and sometimes in prolonged illness, the process stops and the tubules contain only spermatogonia and Sertoli cells. In senility the fibrous tissue increases and the seminiferous tubules may then contain nothing but Sertoli cells.

Deficiency of vitamins A and E also cause atrophy of the seminiferous tubules, just as radiation doses may cause the loss of the germinal epithelium.

Prostate. Before puberty the organ consists chiefly of blindly ending ducts, embedded in connective tissue that contains much smooth muscle. At puberty the typical alveolar system develops, chiefly by hyperplasia of the epithelium of the ducts and their end buds. In old age the prostate tends to undergo hyperplastic enlargement; both glandular and inter-glandular tissue may be involved and compression of the urethra may result with consequent difficulty in micturition. Concretions in the lumen of the alveoli are common in old age, and probably represent a later stage of the colloidal masses usually present.

Penis. In the young the corpora cavernosa are not developed.

(2) Undescended Testis

If the testis does not undergo the usual descent into the scrotum it does not undergo full development: mature spermatozoa are not produced, some of the tubules contain only Sertoli cells and spermatogonia, and the whole testis remains small in size.

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CHAPTER 24

FEMALE REPRODUCTIVE ORGANS

The organs of reproduction in the female include the paired ovaries, the ducts (uterine or Fallopian tubes, uterus and vagina), the external genitalia (clitoris, labia), and the mammary glands.

Most of these structures undergo marked structural changes during the reproductive cycle: these changes are mentioned in relation to each organ.

THE OVARY (Fig. 24.1)

The ovary is a solid organ attached by its antero-lateral border to the broad ligament. In the region of attachment (the *hilum*) are found smooth muscle fibres and numerous large blood vessels and lymphatics; the tissue of the broad ligament is continued into the ovary, where it spreads out as the *stroma*. The free surface of the ovary is covered by a modified peritoneum,

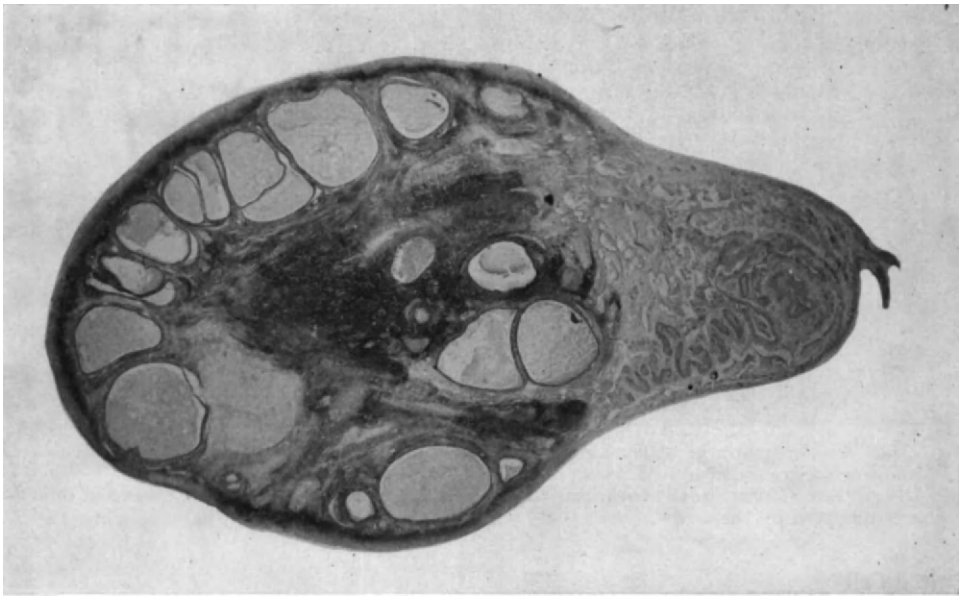

FIG. 24.1. A section of the entire ovary. Note the scattered Graafian follicles and the dense connective tissue of the stroma. The hilum, with numerous sectioned blood vessels, is at the right. 0·25 μm.

the usual flat mesothelial cells being replaced by a layer of cubical cells (known unfortunately as the *germinal epithelium*); the sub-peritoneal connective tissue is continuous with the ovarian stroma. Immediately beneath the epithelium the stroma is condensed to give a *tunica albuginea*. Scattered throughout the stroma are vesicles of different sizes: these are the developing *Graafian* (or *ovarian*) *follicles* (Fig. 24.2). Also scattered through the stroma are groups of large interstitial cells.

Stroma

In the human ovary the stroma consists of elongated, fusiform, connective tissue cells supported by a framework of reticular fibres and also containing elastic networks near the hilum. The outer or cortical part of the stroma in which the follicles are embedded is more cellular than the central medullary portion. The stroma surrounding the developing follicles is differentiated into a *theca* or sheath: at first the stretching due to the growth of the follicle results in a concentric arrangement of spindle-shaped cells and fibres round the basal lamina of the follicle; later two layers are formed by an increase of capillaries in the inner layer and a growth of connective tissue cells on the periphery.

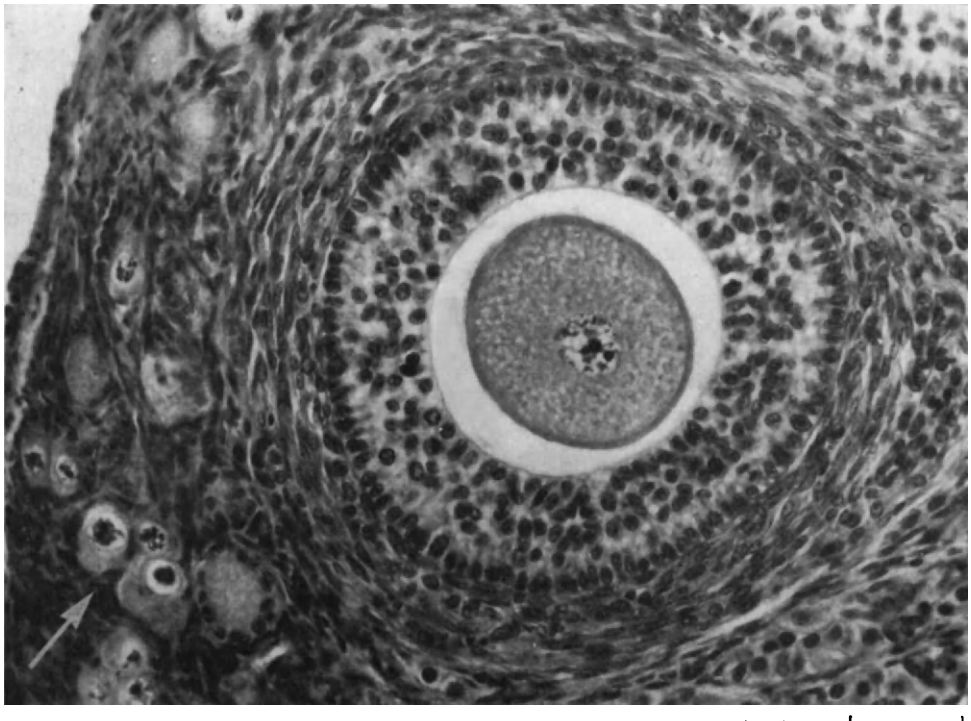

FIG. 24.2. A micrograph of a developing Graafian follicle. The oöcyte with its nucleus is surrounded by the zona pellucida and may be seen in the centre of the picture. Concentrically-arranged thecal cells are also visible. Several primordial follicles are indicated by the arrow. 50 μm.

Interstitial Cells

In the human adult the interstitial cells are not well developed. Typically the cells are large, epithelioid, polyhedral and eosinophil; they contain lipid droplets and are scattered in islands through the stroma. These cells are best developed during the first year of life: they are derived largely from the theca of atretic follicles (see p. 379), and atresia of the follicles is most in evidence at this time. In some adult mammals, e.g. rabbit, the cells are very well developed.

Development of the Graafian (Ovarian) Follicle

The oöcytes in the ovary are all derived originally from the primordial germ cells which appear early in the development of the embryo and migrate from the region of the yolk sac wall into the developing gonad. At a very early foetal stage the epithelial cells covering the

developing gonad proliferate and form cell cords which grow inwards into the stroma of the ovary; these are known as the *medullary* or *sex cords*. The cords lose connection with the surface and become surrounded by stromal cells forming nests of cells each containing one of the invading primordial germ cells which becomes rather larger than the remaining cells of what are now termed *primordial follicles* (Fig. 24.3). These primordial follicles are found in the greatest number in the periphery of the ovary and as these are the source of all mature follicles, it follows that their number diminishes with age.

During the early years of life the primordial follicles remain quiescent in the ovary but at the onset of puberty with the commencement of monthly ovulation several follicles each

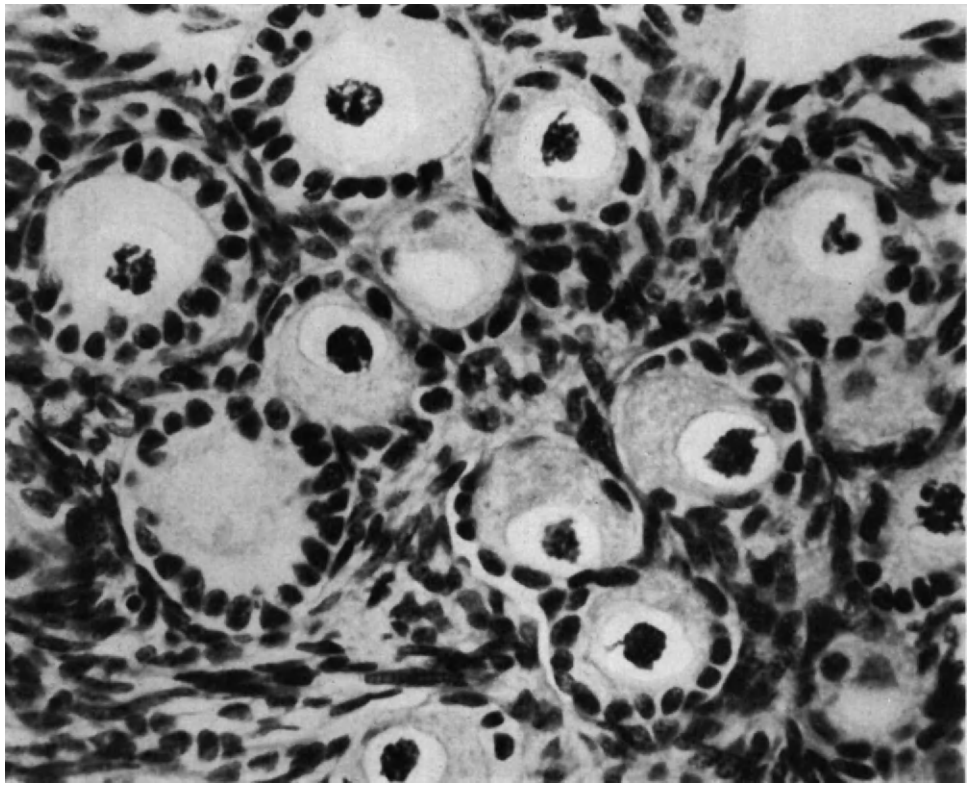

FIG. 24.3. A clump of primordial follicles. Note the central single germ cell in each follicle surrounded by a single layer of follicular cells. 10 μm.

month begin to develop further, although usually only one reaches maturity and actually discharges its ovum from the ovary. During the development of a follicle there is firstly an increase in size to about 40 μm. in diameter, followed by a proliferation of the epithelial-like cells which surround the germ cell. These cells assume a columnar form and soon several layers may be distinguished; at this stage these cells may be termed the *granulosa cells* (Figs. 24.2, 24.4). They are rich in mitochondria, granular endoplasmic reticulum and free ribosomes. The surface of the granulosa cells which is apposed to the developing oöcyte has been shown by the E/M to possess microvilli which penetrate into the so-called *zona pellucida*, a fibrillar layer of mucoprotein which surrounds the oöcyte. The zona pellucida (Figs. 24.4, 24.5) is probably secreted both by the oöcyte itself and by the granulosa cells, although the details of the process are not clear.

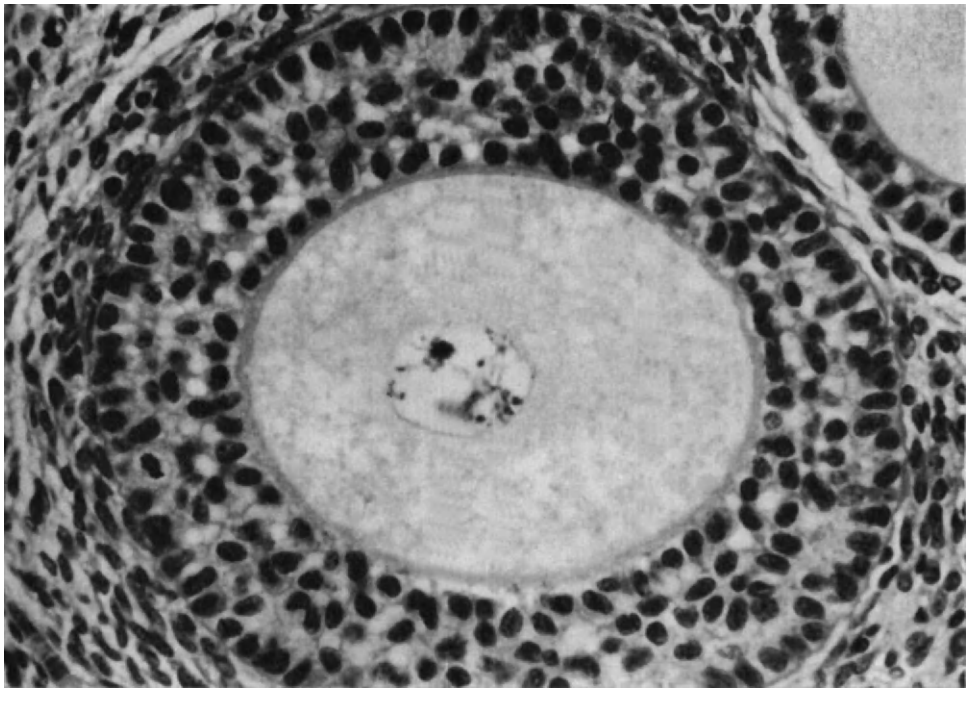

FIG. 24.4. Granulosa cells surrounding a developing follicle. Note also the zona pellucida and the large oocyte nucleus with prominent nucleolus. 15 μm.

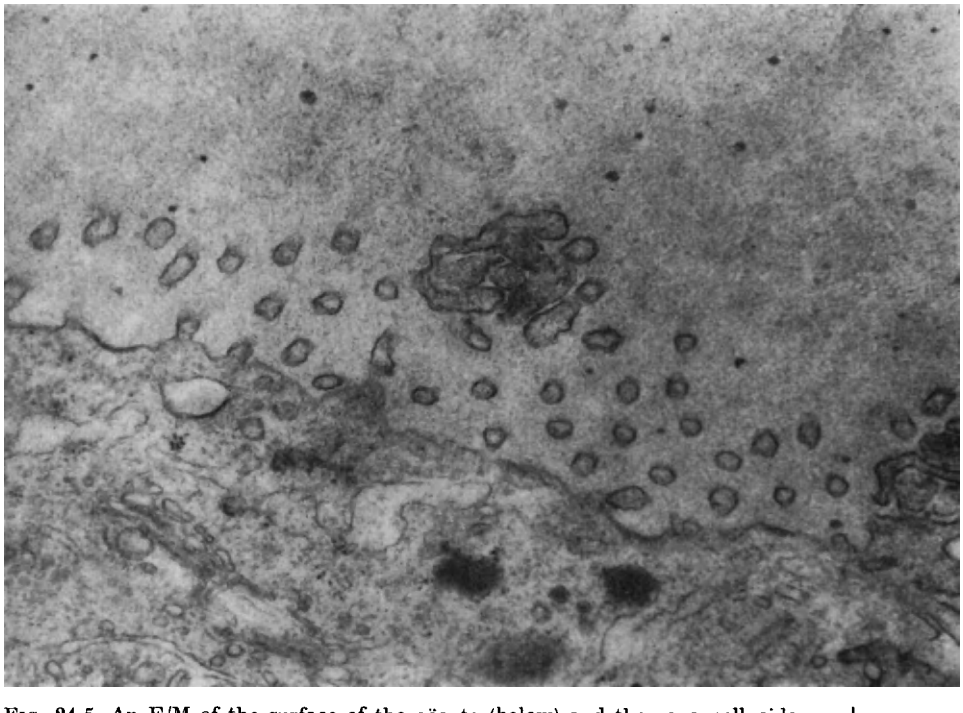

FIG. 24.5. An E/M of the surface of the oöcyte (below) and the zona pellucida. The short microvilli of the oöcyte surface penetrate into the substance of the zona pellucida. 0·5 μm.

As growth of the follicle proceeds, several irregular spaces containing fluid appear between the granulosa cells and eventually become confluent. At this stage the oöcyte is then attached by a mass of cells (the *cumulus oöphorus* or *discus proligerus*) to the inner wall of the large follicular cavity and projects into it (Fig. 24.6). The cavity is filled with the *liquor folliculi* which is also rich in mucoproteins. Outside the granulosa layer the stroma has been modified to form a *theca*, separated from the granulosa cells by a prominent basal lamina, sometimes termed the *glassy membrane*.

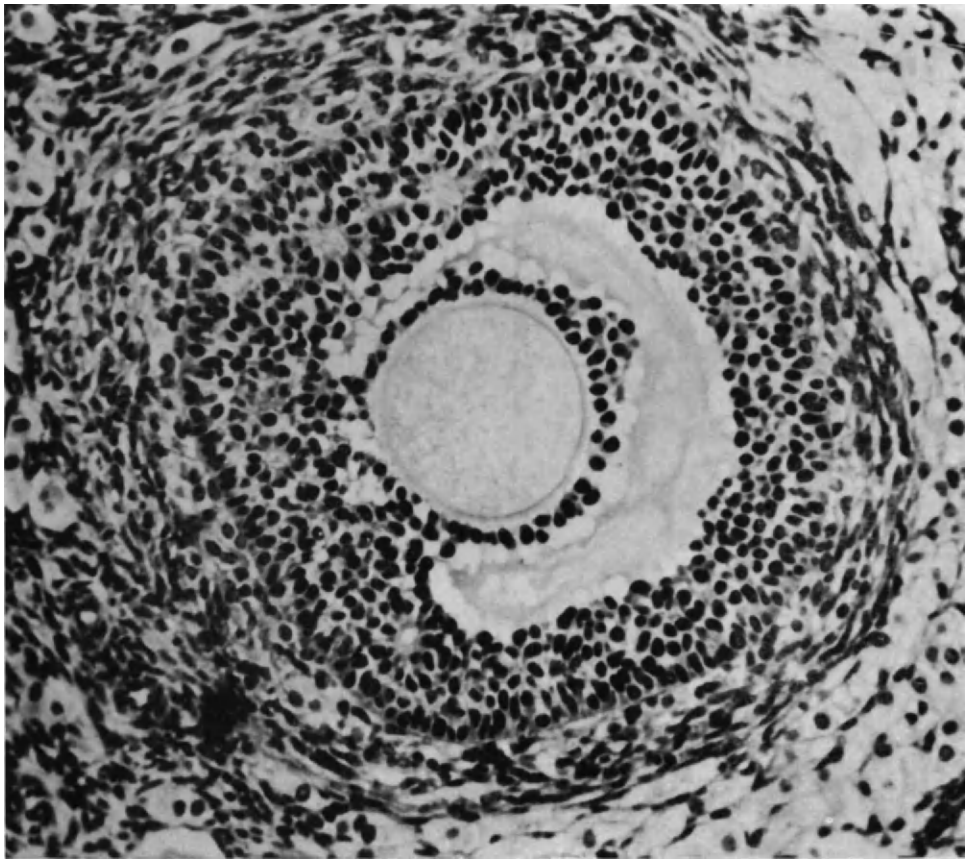

FIG. 24.6. A nearly mature Graafian follicle with the oöcyte embedded within the discus proligerus. The zona pellucida, coagulated liquor folliculi and the thecal cells are all clearly visible. 50 μm.

As the oöcyte matures, the theca clearly differentiates into concentrically-arranged fibres and fusiform cells—the *theca externa*—which is richly vascularized. Inside the theca externa the cells appear large and ovoid or spindle-shaped and are known as the *theca interna*. This latter layer is thought to secrete oestrogens and its cells show the typical features (for example, smooth endoplasmic reticulum) associated with cells secreting steroids. There is also a rich capillary plexus in the theca interna. During this differentiation of the follicle, the whole mass has sunk further into the substance of the ovary. When mature the follicle either ruptures or it may undergo involution: occasionally the follicles develop into cysts. If the follicle is going to rupture the amount of fluid increases greatly and the follicle moves to the surface of the ovary. During the final phase of follicular maturation a small area of the wall of the follicle, together

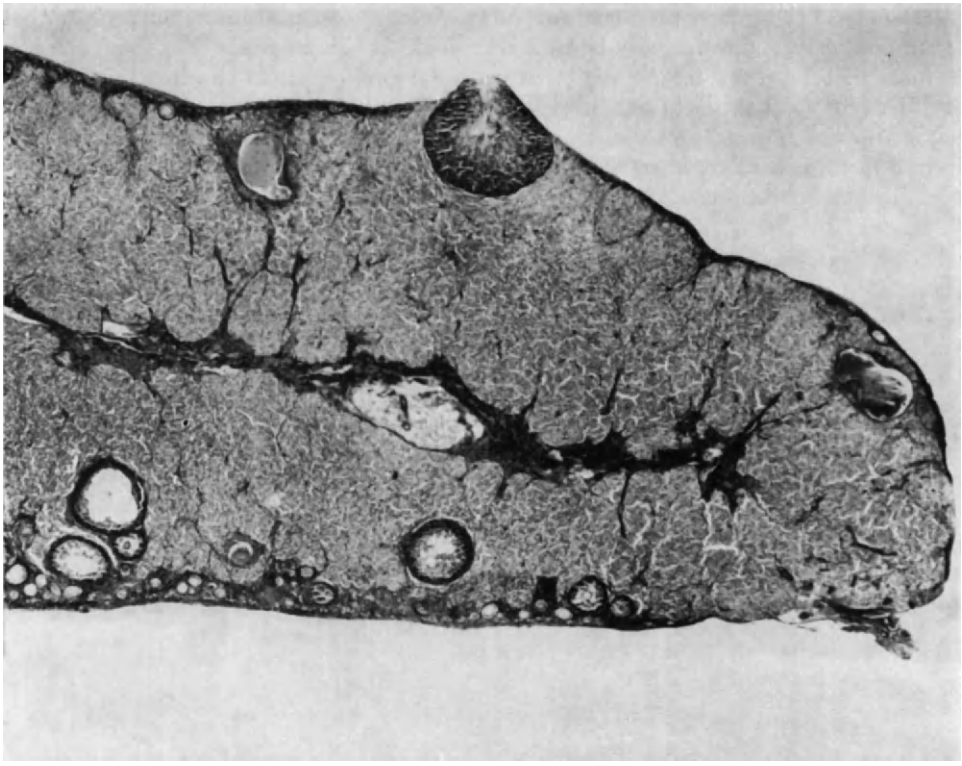

FIG. 24.7. A section of a rabbit ovary showing several maturing follicles and one which has just ruptured. Micrograph by Dr. H. M. Charlton.

0·1 cm.

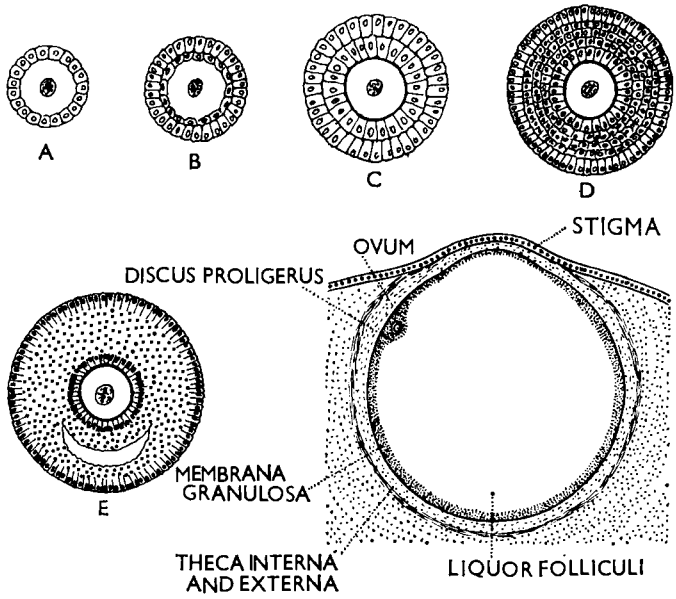

FIG. 24.8. A diagram of the ovarian cycle summarizing the morphology of the Graafian follicle at various stages of its development (A—E) and when mature.

with the overlying ovarian tissue, becomes thin and translucent, forming the *stigma*, before ovulation this protrudes and forms a small blister on the surface of the ovary. The stigma tears open and the ovum, surrounded by discus proligerus cells (forming the *corona radiata*), is set free with the liquor folliculi, oozing slowly out into the peritoneal cavity: this process is known as *ovulation* (Fig. 24.7). The corona radiata is usually regarded as both being protective and nutritive for the discharged ovum. The fimbriae of the oviduct (p. 381) probably closely invest the ovary at the time of ovulation. In the cavity left in the substance of the ovary the corpus luteum is developed (see below). One follicle usually ripens every twenty-eight days.

The process of follicle maturation is summarized diagrammatically in Fig. 24.8.

The Ovum

The ovum is a large spherical cell which when mature may have a diameter of over 100 μm. It contains yolk granules (although these are few in the human in comparison to other species) and a large nucleus (the *germinal vesicle*) with a well developed nucleolus. In the early stages of development there is a single Golgi apparatus near the nucleus, but as the ovum develops numerous Golgi complexes appear and become scattered throughout the cytoplasm. At the same time there is a marked increase in the amount of the rough-surfaced endoplasmic reticulum and free ribosomes in the cytoplasm. The ovum is surrounded by a clear membrane or zona pellucida which is composed of highly sulphated mucoproteins. In the E/M this membrane appears to have a fibrillar structure and is penetrated by numerous long, often branched microvilli from both the granulosa cells and from the surface of the ovum itself (Fig. 24.5).

The primitive (or primordial) ova correspond to the primary spermatocyte stage in the development of the sperms (p. 357): they can be called accordingly *primary oöcytes*. In the two subsequent divisions one daughter cell receives nearly all the cytoplasm, the other being very small and known as the *polar body*. In this way *secondary oöcytes* are formed, a reduction of the somatic number of chromosomes to the haploid number having been effected during maturation. The first polar body is formed while the ovum is in the Graafian follicle and the second after it has been set free as an oöcyte in the metaphase of the second meiotic division.

The Corpus Luteum

After a ripe follicle has ruptured and the ovum has been discharged, the remains of the follicle are transformed into a corpus luteum (Fig. 24.9). The cavity contains at first some follicular fluid and blood: the wall collapses, so that the lining membrana granulosa is thrown into folds. These cells rapidly increase greatly in size and are piled up in layers that become drawn out radially: the cells of the theca interna remain at the periphery: connective tissue from the theca externa pushes into the cell mass carrying capillaries. In this way the periphery of the cavity is quickly filled up with columns of large cells containing yellowish lipid material (lutein). The cells appear very vacuolated in the optical microscope (Fig. 24.10) and with the E/M large amounts of smooth surfaced endoplasmic reticulum and lipid droplets may be seen. Between the cell columns are fibrous trabeculae with many sinus-like capillaries, while the centre of the mass is occupied by loose connective tissue (see Fig. 24.9). Cells from the theca interna may also become "luteinized" and contribute to the corpus luteum.

Further changes in the corpus luteum depend on the fate of the discharged ovum. If the ovum has perished on its way to the exterior the corpus luteum survives for about 14 days and then degenerates; this is the *corpus luteum of menstruation*. If the ovum has been fertilized the corpus luteum enlarges and persists during pregnancy as the *corpus luteum of pregnancy*: it may then reach a diameter of 2 cm.

If fertilization of the ovum has not taken place slight haemorrhage into the corpus luteum occurs at the next menstruation and this is the beginning of its involution: the cells show increasing infiltration with fat and shrink, while the blood clot is organized by the connective

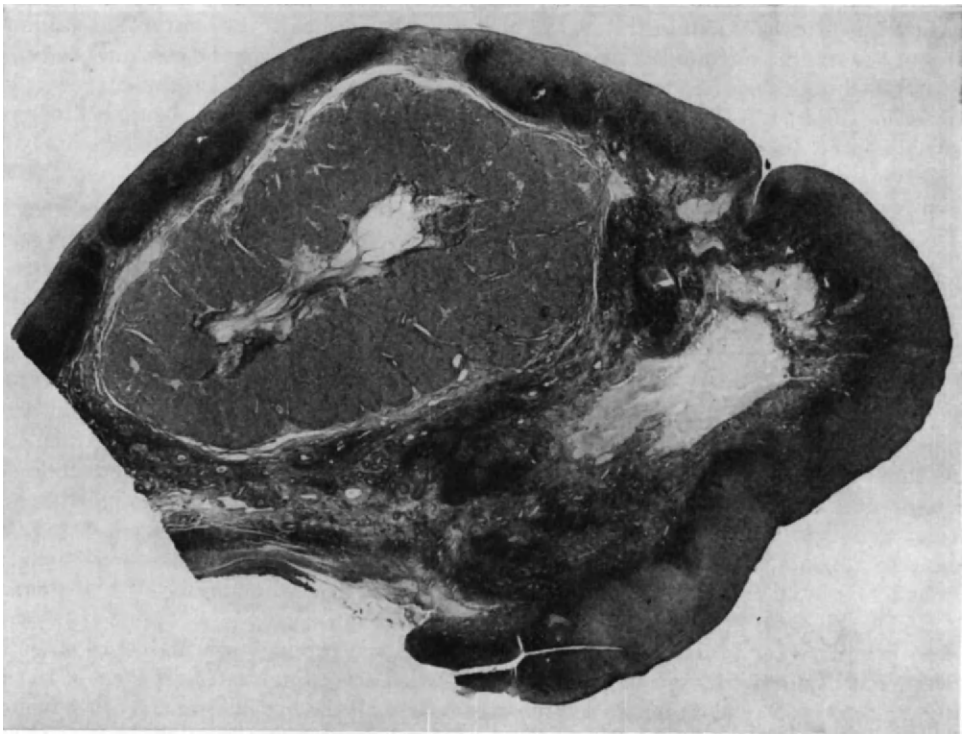

FIG. 24.9. A low power micrograph of an ovary containing a single large corpus luteum and some corpora albicantia. The luteal cells of the large corpus luteum surround a central mass of loose connective tissue. 0·25 cm.

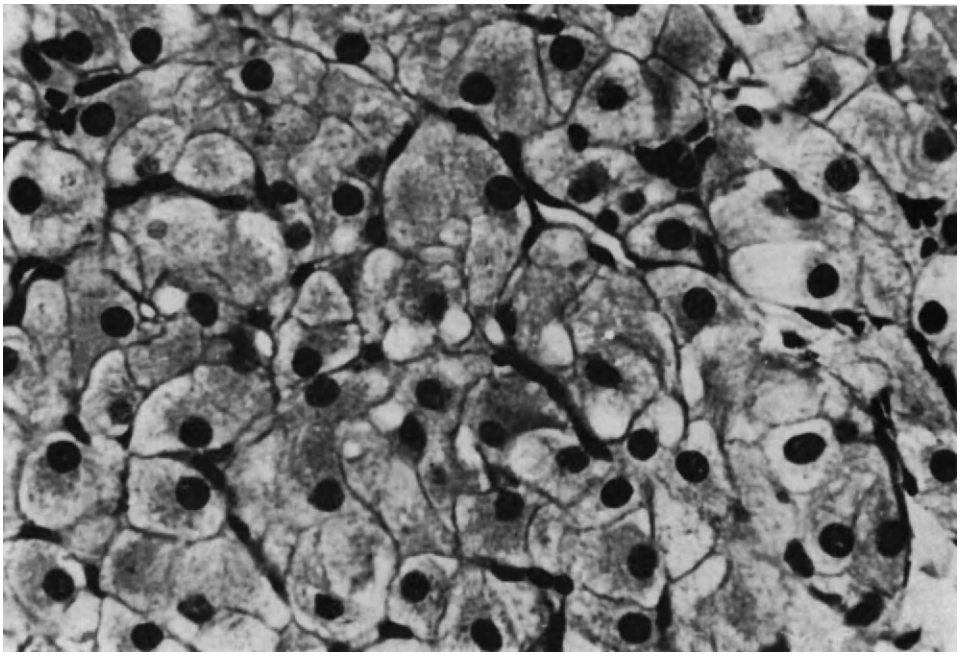

FIG. 24.10. Luteal cells at high magnification; the "empty" vacuolated appearance of the cytoplasm is due to solution of the steroids from the cells during the preparation of the section. 10 μm.

tissue: the whole structure shrinks, and after about three months is represented by a fibrous hyaline scar, the *corpus albicans* (Fig. 24.9).

If fertilization of the ovum takes place menstruation is suppressed and consequently no haemorrhage into the corpus luteum occurs. The luteal mass continues to grow until about the fifth month of pregnancy, when involution begins. Involution is much accelerated after parturition, and finally a corpus albicans is produced. A corpus luteum may sometimes give rise to a cyst.

Atresia (involution) of Follicles

Baker has shown that each human ovary contains at least 7 million germ cells by the middle of gestation; this number declines to about 2 million at birth. As normally only one ovum is discharged at every menstrual period during the usual thirty years of sexual activity following puberty there remain many hundreds of thousands of germ cells to account for: these disappear gradually by degeneration (atresia). This process has already begun in the infant, and is very marked just before puberty: it is completed after the menopause, so that every ovary contains some degenerating follicles. Degeneration of the follicle may occur at any stage of its development, the ovum itself being first affected. In atresia of a primary follicle the ovum degenerates, followed by the follicular cells, and the space is completely filled by stroma. If the follicle is growing, first the ovum undergoes fatty degeneration followed by the follicular cells: the zona pellucida becomes folded, and the theca interna cells enlarge, and the basal lamina is transformed into a thick layer of hyaline substance. This glassy membrane persists as the follicular cells disappear, and the theca cells are replaced by fibrous tissue, with the remains of the folded pellucida membrane which may persist for some time. Ultimately the follicle is replaced by fibrous tissue often with a glassy streak: this ultimately disappears in the stroma of the ovary.

Blood Vessels and Lymphatics

The arteries enter at the hilum, branch and run a tortuous course through the substance of the ovary: their structure is similar to that of the helicine arteries of the penis. Plexuses are formed which give rise to vascular networks in the theca of the Graafian follicles and also supply the corporea lutea. The veins run with the arteries; they are very large and form a plexus at the hilum. The lymph capillaries arise chiefly in the theca of the Graafian follicles: large lymphatics leave the organ at the hilum.

Nerves

The nerve fibres, mostly non-myelinated, enter at the hilum with the blood vessels. Many of these fibres supply the muscle of the blood vessels, but a few penetrate to supply the follicles and the germinal epithelium: in some parts there is a very close relation between the nerve fibres and groups of paraganglionic cells that appear somewhat like interstitial cells (see p. 372). Sensory fibres arising from Pacinian corpuscles are sometimes present in the stroma.

The Ovarian Cycle (Fig. 24.11)

This consists of (1) a gradual ripening of a Graafian follicle (commencing at menstruation, see p. 373) culminating in liberation of the ovum about the middle of the intermenstrual period, and (2) the formation immediately after ovulation of the corpus luteum that lasts until the next menstrual period. If fertilization occurs menstruation ceases, and the corpus luteum persists throughout pregnancy.

These changes are controlled by the pars distalis of the adenohypophysis which secretes two gonadotrophic hormones, "follicle stimulating hormone" (F.S.H.) and "luteinizing hormone" (L.H.). At the beginning of the menstrual cycle the blood levels of FSH and LH start to rise, due to activity of the pars distalis of the pituitary. In consequence one of the primordial oöcytes in the ovary begins to develop and increase in size. Its theca interna cells

proliferate and secrete increasing amounts of oestrogens (especially oestradiol 17β) into the blood. This rise in plasma oestrogen levels causes proliferation of the uterine endometrium and its glands but, at the same time, also begins to act as a negative feed-back mechanism on the hypothalamus and pituitary so depressing the secretion of FSH. The rising oestrogen concen-

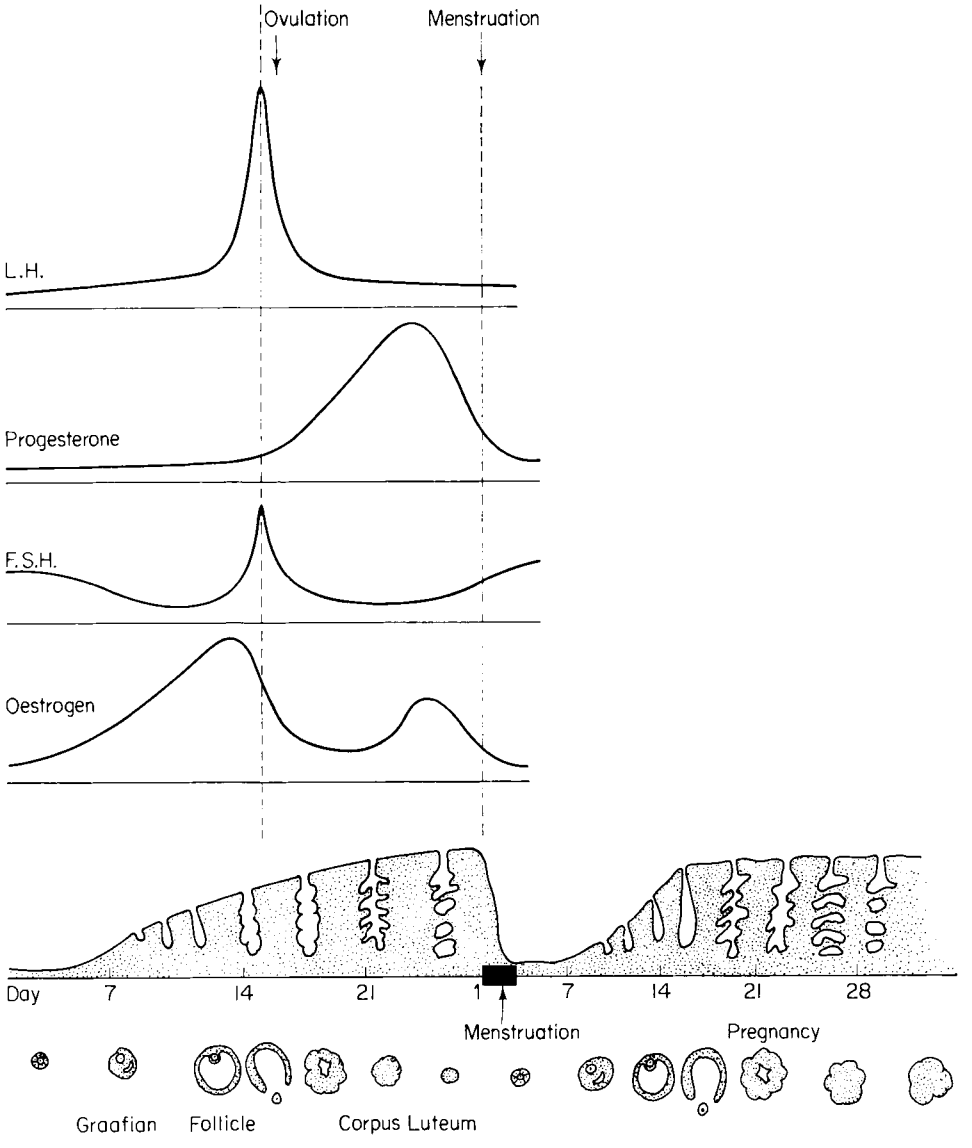

FIG. 24.11. A diagram summarizing the follicular changes, condition of the uterine endometrium and the concentrations of oestrogen, progesterone, FSH and LH in the blood during the menstrual cycle.

tration in the blood eventually triggers off a sudden burst of FSH and LH secretion by the pars distalis and it is this which seems to signal the liberation of the oöcyte from the Graafian follicle. Just before the actual liberation of the oöcyte there seems to be a fall in the rate of oestrogen secretion.

Following ovulation, the remnants of the follicle change into a corpus luteum which commences the secretion of large amounts of progesterone (together with 17α hydroxyprogesterone and oestradiol 17β) into the blood stream so that during the second half of the menstrual cycle there is a rise in the plasma levels of all these steroids. The joint action of oestrogen and progesterone on the endometrium (already primed with oestrogen alone) results in further proliferation of the endometrium and the initiation of its glandular secretion. After ten days or so the corpus luteum begins to regress spontaneously—the exact reason for this still seems uncertain—so that the hormone supplies to the endometrium gradually fail. One

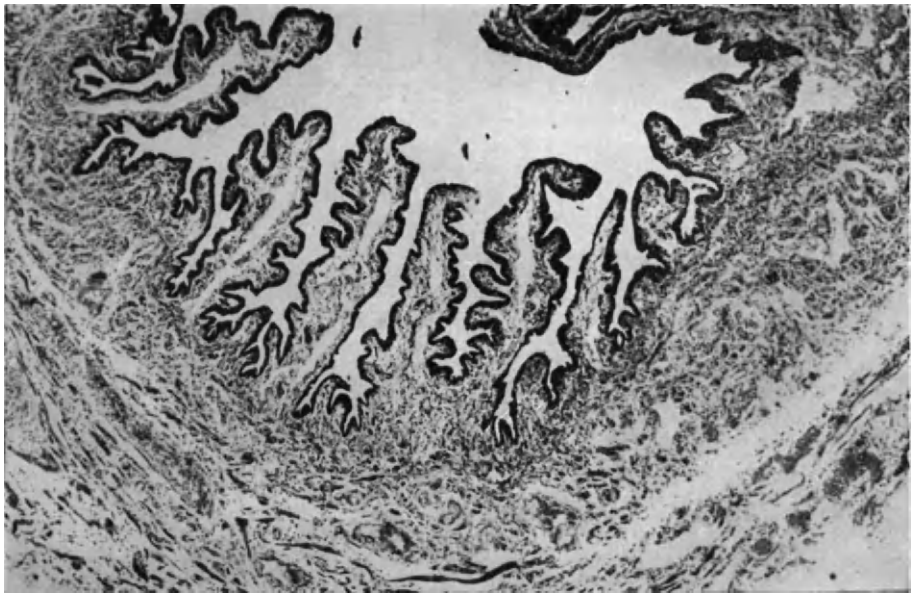

FIG. 24.12. Transverse section of part of the Fallopian tube to show the fimbriae. 100 μm.

consequence of this is that the endometrial arteries eventually go into spasm, thus initiating the menstrual flow of both sloughed off endometrium and blood. At the same time the regression of the corpus luteum lifts the inhibiting negative feedback from the hypothalamus so allowing the pituitary secretion of FSH and LH to recommence and begin a new cycle. The changes in plasma hormone levels and the uterine endometrial thickness are summarized in Fig. 24.11. It will be seen that the cycle depends on the changes in the ovary itself, but that these are intimately related to the changes in the amount of hormones produced by the adenohypophysis.

THE UTERINE (FALLOPIAN) TUBES

The uterine (Fallopian) tube or oviduct consists of (1) an abdominal part (the *ampulla*), that has a wide lumen and a funnel-like opening split into fringes (fimbriae); and (2) a narrow isthmus that extends towards the uterus and then traverses its wall to open into its lumen. The whole tube is covered by peritoneum.

The *muscular coat* consists of an outer, thin longitudinal, and an inner circular layer of smooth muscle: the outer coat is usually lacking at the fimbriated end of the tube.

The *mucous membrane* is thrown up into numerous longitudinal branching folds, particularly well developed near the free opening (Fig. 24.12), where some of the fimbriae extend freely as a

fringe towards the ovary. The epithelium is columnar, consisting of two kinds of cells (Fig. 24.13). Most of the cells in the upper part of the duct are ciliated, beating towards the uterus: the other cells are not ciliated but contain granules and are glandular in nature. The underlying stroma consists of a cellular connective tissue without glands.

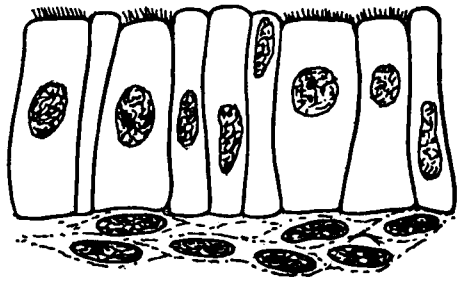

FIG. 24.13A. Diagram of the two cell types in the epithelium of the Fallopian tube. The cells which lack cilia are glandular cells.

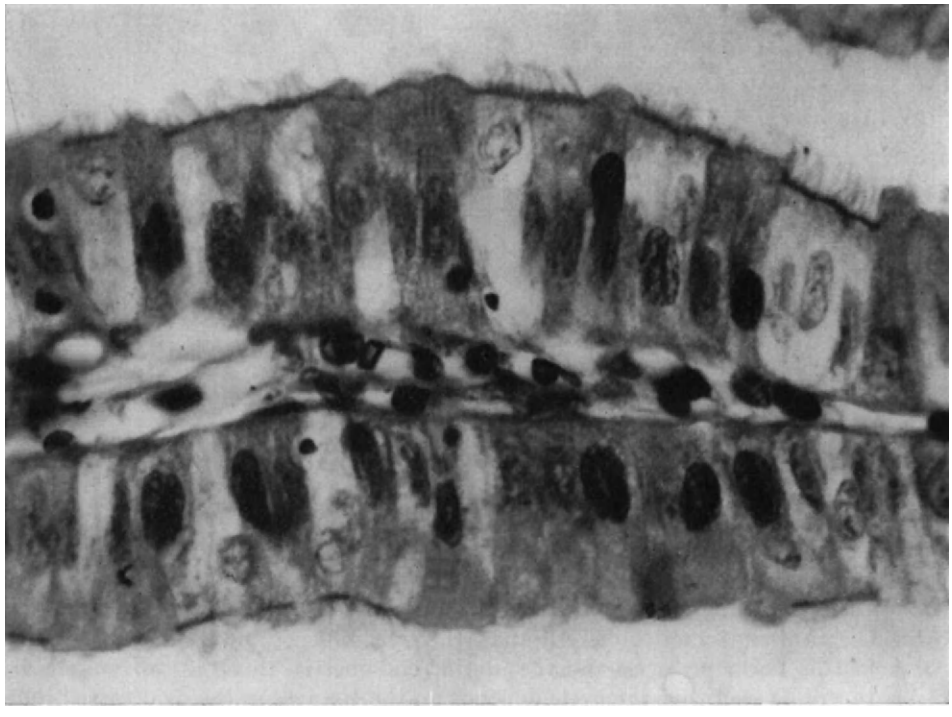

FIG. 24.13B. Photomicrograph of the epithelium of a fimbria of the Fallopian tube to show the two types of cell in the epithelium. Note the stroma between the two epithelial layers on opposite sides of the fimbria. 10 μm.

Physiological Variations in Structure

During pregnancy the epithelial cells become lower and the glandular cells become more granular. The cilia disappear after the menopause. Blandau suggests that in monkeys there may also be a regression of cilia during the luteal phase of the cycle. The ciliary activity, coupled with peristaltic movements of the tube musculature seem to control the movement of the ovum or zygote down the Fallopian tube.

Blood Vessels and Lymphatics

The serous covering and the mucous membrane are both well supplied with blood vessels and with lymphatics. At its abdominal opening the oviduct possesses a ring of large veins in the

mucosa between and in the fimbriae, with a network of muscle fibres between them: this provides a kind of erectile tissue. At the time of ovulation the vessels are filled with blood, so that the fimbriae become enlarged and turgescent, coming into contact with the surface of the ovary.

Nerves. Plexuses of nerve fibres supply the muscle and the stroma.

THE UTERUS

This pear-shaped organ (Fig. 24.14), with its thick muscular wall, consists of four parts: (1) the *body* with its rounded upper end (*fundus*), (2) the *isthmus*, (3) the *cervix* or cylindrical lower part, (4) the *portio vaginalis*, pierced by the cervical canal and protruding into the vagina. The wall of the uterus consists of three layers, the serous, muscular and mucous coats, the latter being more usually known as the *endometrium*.

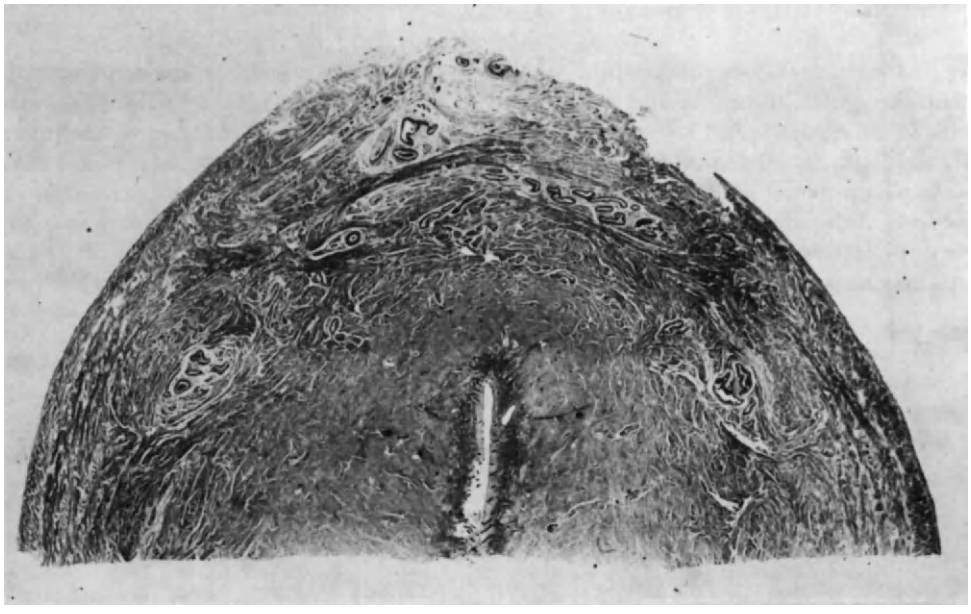

FIG. 24.14. A transverse section of a uterus, which has been bisected in the sagittal plane. The serous coat is very thin, the bulk of the wall being myometrium. The endometrium is the darkly staining tissue surrounding the slit-like uterine lumen in the middle of the picture. 0·25 cm.

(1) *Serous Coat.* This is derived from the peritoneum, and covers the greater part of the fundus: it has the usual structure of peritoneum.

(2) *Muscular Coat* (*or Myometrium*). This is a thick coat; the various layers are not sharply differentiated and contain a considerable amount of interstitial connective tissue. The outer layer is thin and the fibres are arranged both longitudinally and circularly. The middle layer is thick, the fibres are for the greater part circular with a few running obliquely, and it contains many large blood vessels, chiefly veins. The inner layer is thin and the fibres are both longitudinal and circular, the latter forming sphincters round the oviducal openings. In the cervix of the uterus most of the muscle fibres are circularly arranged. The connective tissue between the bundles consists of collagen and elastic networks, and contains fibroblasts, macrophages, mast cells and immature connective tissue cells.

In the cervix the collagenous and elastic fibres are especially dense, accounting for the firm consistency of this part of the uterus.

In pregnancy the muscle cells increase in length from their usual average of 50 μm. to 500 μm. or more (Fig. 24.15), and their number is also increased by transformation of the immature connective tissue cells into new muscle fibres. At the same time the connective tissue increases and so loosens the muscle bundles. After parturition the muscle fibres undergo fatty change and diminish in size: some of them degenerate.

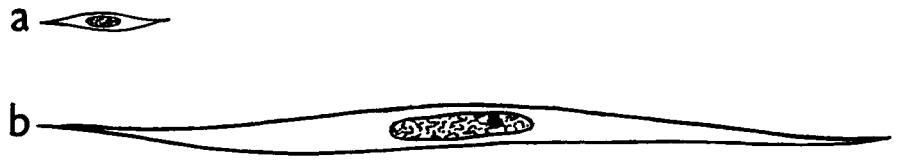

FIG. 24.15. a, normal smooth muscle cells of the myometrium; b, hypertrophied myometrial cells in the later stages of pregnancy.

(3) *Endometrium*. The endometrium consists of a connective tissue stroma composed of fine fibrils and irregular branching and spindle-shaped cells resembling mesenchyme. These make a network in which are found leucocytes of all types. It is lined by a columnar epithelium containing small groups of ciliated cells interspersed with secretory cells. Near the cervix it changes into a simple columnar and then to a stratified epithelium. The columnar epithelium dips down to line long, tubular convoluted glands that extend through the mucous membrane to the muscle coat. In the *cervix* the mucosa is thicker and less cellular than in the body of the uterus. The epithelium of the lining and of the glands is high columnar and secretes mucin. Frequently

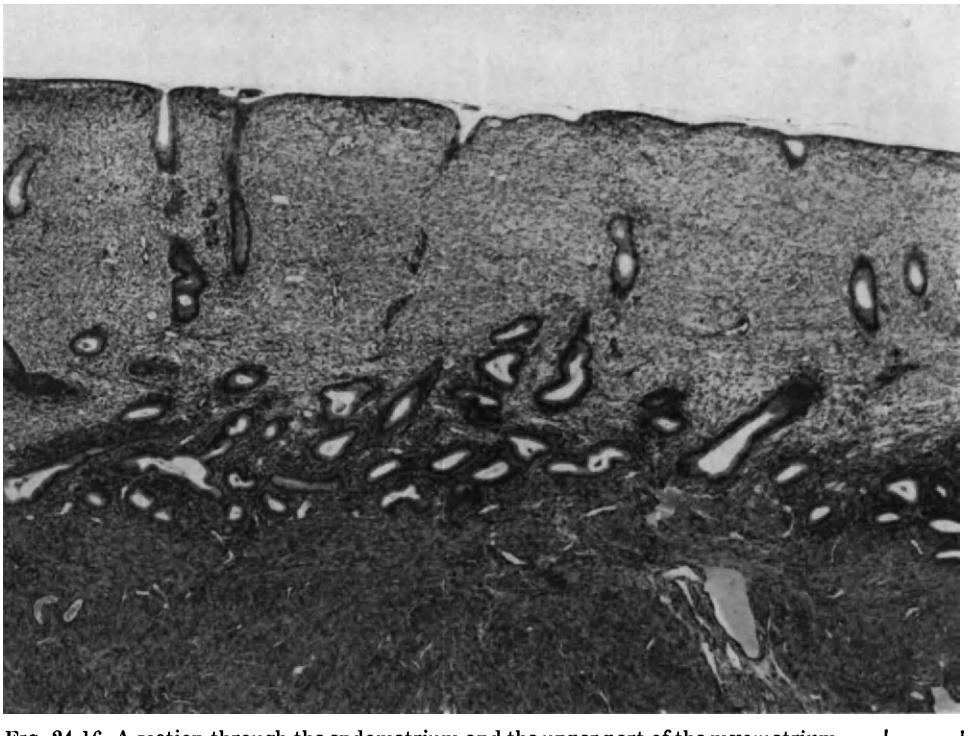

FIG. 24.16. A section through the endometrium and the upper part of the myometrium in the early proliferative phase of the cycle. Note the thickness of the mucosa and the simple coiled uterine glands. 1 mm.

the gland mouths are blocked so that *retention cysts* (or Nabothian follicles) occur. The whole endometrium is highly vascular and contains many lymphatics.

The vascular supply of the endometrium plays a very important role in the changes associated with menstruation (see below). The more superficial part of the endometrium is supplied by *spiral arteries* arising from the middle layer (stratum vasculare) of the myometrium. The deeper part of the endometrium which is unchanged during the menstrual cycle, has a separate blood supply.

Physiological Changes in the Uterine Endometrium during Sexual Activity

The endometrium of the uterus undergoes pronounced changes during the menstrual cycle and in connection with pregnancy.

(*a*) **Changes During the Menstrual Cycle.** Taking the menstrual cycle as twenty-eight days, the changes in the endometrium consist of a *reparative* stage immediately following menstruation (four to five days), a *proliferative* stage (Fig. 24.16) (about ten days) during which the

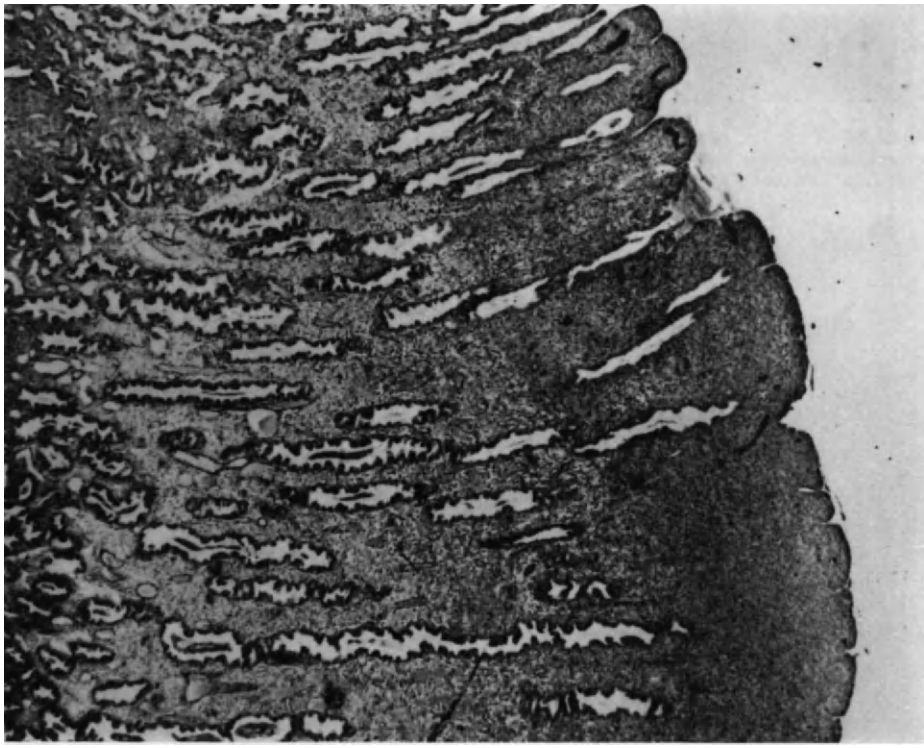

FIG. 24.17. A section through the endometrium in the late secretory phase. The mucosa is now much thicker and the glands have become "sawtoothed" and contain secretion in their lumen; the stroma is also more oedoematous. 1 mm.

mucosa thickens enormously, a *secretory* or *luteal* stage (Fig. 24.17) (about ten days) during which vascular engorgement and secretion take place, and a *destructive* stage (about four days) during which the menstrual flow occurs. During the proliferative and secretory phases, there is a continuous increase in the amount of glycogen and mucin within the cells of the uterine glands. In the secretory phase the epithelial cells lining the glands become elongated and have ragged-looking free borders. The epithelium becomes folded in such a manner as to give a

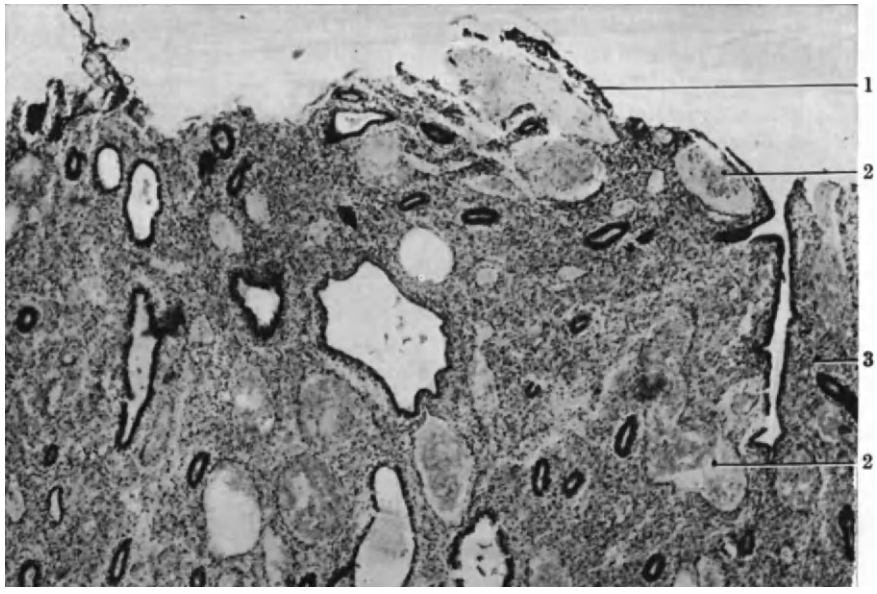

FIG. 24.18. A section through the endometrium in the menstrual stage. 1, epithelium which is in the process of sloughing off; 2, dilated blood vessel; 3, cellular stroma. 1 mm.

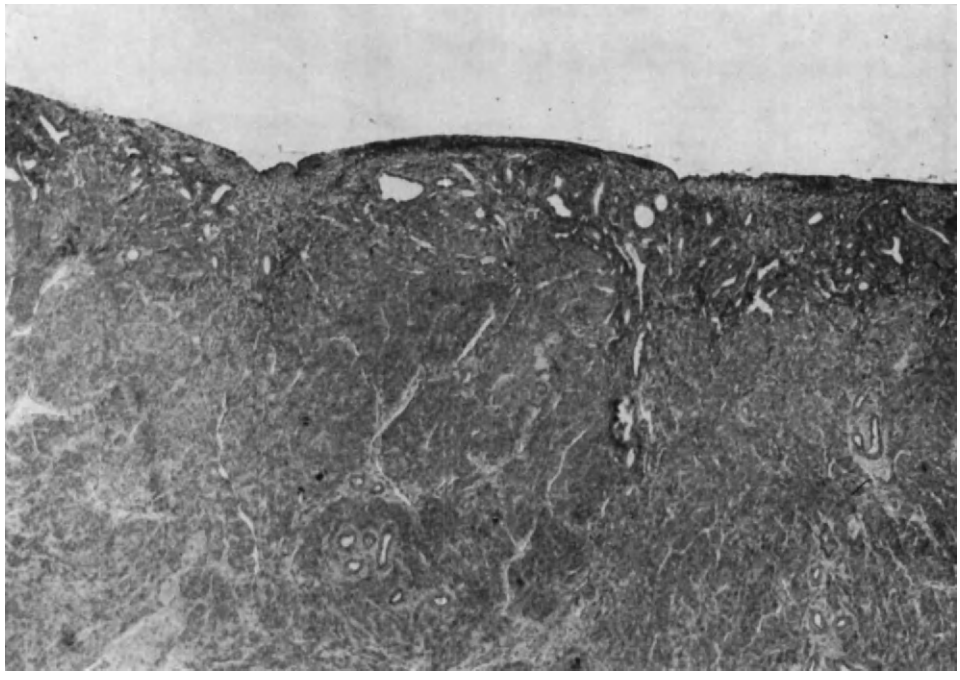

FIG. 24.19. Section through the endometrium after the menopause; the glands are now barely visible and the endometrium is very thin. The bulk of the tissue in the picture is myometrium. 1 mm.

characteristic "saw-toothed" appearance when seen under medium powers of the microscope (Fig. 24.17).

Just before menstruation begins, the arteries to the more superficial part of the endometrium constrict for considerable periods at a time. Their coiled nature increases the efficiency of the vaso-constriction, so that the tissues supplied by them become markedly ischaemic and begin to undergo degenerative changes. When the vessels dilate again, the rush of blood bursts through the damaged walls of the smaller blood vessels and so into the surrounding tissue. As this process is repeated, areas of endometrium break off, and pass along with blood, into the uterine cavity (Fig. 24.18). Ultimately the whole of the endometrium supplied by the coiled arteries is sloughed off in this way. Menstruation occurs about fourteen days before ovulation.

As soon as the disintegrated tissue has been shed the uterine arteries constrict and epithelial cells from the open mouths of the glands spread over the bare surfaces of the connective tissue and rapidly cover them: the cells of the connective tissue and of the glandular epithelium multiply and the mucosa is quickly restored to its resting condition.

(*b*) **Changes During Pregnancy.** If pregnancy occurs, the secretory phase of the endometrium continues and the endometrium in certain parts becomes greatly thickened and transformed into the *decidua*, in which the fertilized ovum is buried. In the decidua the stromal cells are changed into large epithelial cells of peculiar type called *decidual cells*. These are frequently pointed at one end, have a large spherical nucleus usually at one pole of the cell, and a very finely granular cytoplasm: they contain glycogen and towards the end of pregnancy they contain brown pigment. The glands are elongated and become more convoluted. Part of the decidua is differentiated into the maternal part of the placenta.

(*c*) **Changes After Parturition.** Repair occurs in the same way as in the post-menstrual phase.

(*d*) **Changes After the Menopause.** The endometrium becomes very thin (Fig. 24.19): the glands tend to become obliterated and to form cysts.

As the changes in the uterine endometrium are very important, they are summarized for convenience in the following table.

Stage of cycle	*Endometrium*	*Glands*	*Coiled Arteries*
Reparative and Proliferative	1–3 mm., many mitoses	Straight, tall epithelium, many mitoses	Not apparent in superior third
Secretory	3–6 mm., oedematous	Wide lumina, very active, surface "blebbing" of cells. "Sawtooth" appearance of whole gland	Clearly seen near surface
Premenstrual	*c.* 6 mm. thick, very oedematous, leucocytosis apparent	"Sawtooth" and lumina full of secretion	Clearly seen near surface
Menstrual	0·5–1 mm., much extravasation of blood and desquamation of epithelium	Collapsed	Collapsed
Senile	Very thin	Atrophied	Not apparent

Blood Vessels

The large blood vessels run chiefly in between the muscle coats of the uterus. In the myometrium the capillaries have a thick endothelium and a very small lumen, and the large veins form a plexus without any valves. The endometrium has a very rich capillary network, particularly dense just below the surface epithelium.

If a pregnancy has taken place the vessels show permanent irregular thickening of the intima which also contains smooth muscle, and in the media the muscle is almost entirely replaced by elastic networks.

Lymphatics

The lymph capillaries begin in the mucous membrane where they make a network: this communicates with another plexus in the myometrium and drains into large valved vessels in the outer serous coat.

Nerves

Nerve fibres of the autonomic systems supply the muscle, the blood vessels and the glands.

THE VAGINA

The wall of the vagina has three coats (Fig. 24.20).

The external *fibrous coat* consists of connective tissue merging into that of the surrounding structures, often with numerous adipose cells included in it.

The *muscular coat* consists of smooth muscle fibres arranged for the most part in a longitudinal direction: between the muscle bundles is connective tissue containing well-developed elastic networks. The sphincter of the vagina is made up of circularly-arranged striated muscle fibres.

The *mucous coat* consists of a stroma of dense, vascular connective tissue which in parts is thrown up into papillae. Lymph nodules are common and the whole stroma contains many lymphocytes. The epithelium (Fig. 24.21) is of the stratified squamous variety, the superficial cells containing glycogen. There are no glands, the mucus in the vagina originating in the glands of the cervix.

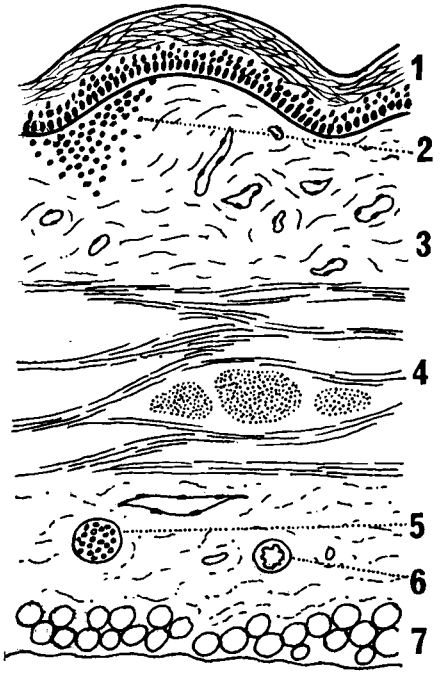

FIG. 24.20. The organization of the vaginal wall: 1, stratified squamous epithelium; 2, lymphoid tissue; 3, stroma of loose connective tissue; 4, muscular coat (chiefly longitudinal); 5, small nerve cells; 6, blood vessel; 7, adipose tissue in the connective tissue coat.

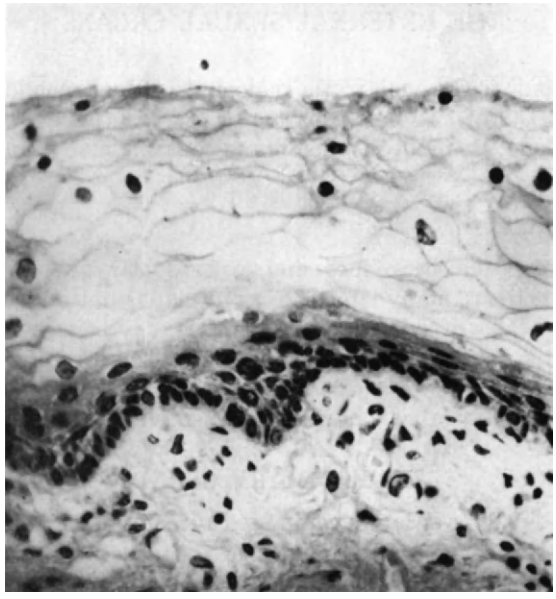

FIG. 24.21. A micrograph of the stratified squamous epithelium of the vagina. 70 μm.

The *hymen*, at the lower end of the vagina, is a thin fold of the mucous membrane, covered on both sides with stratified epithelium.

In the *vestibule* the mucous coat gradually merges into the structure of skin. There are many small mucous glands and also the larger *vestibular glands* (of Bartholin).

Vaginal Changes during the Sexual Cycle

(*a*) **Menstruation.** Eight to ten days before menstruation the surface layers of cells of the epithelium become keratinized: during menstruation the cells lying within this layer are shed. [In some animals, e.g. mouse (but not in primates), the oestrus cycle is characterized by well-marked changes. Before oestrus the epithelium becomes very thick and begins to keratinize: during oestrus the upper cells keratinize and die: after heat the keratinized epithelium is shed and there is a great infiltration with leucocytes. By studying smears of the mucosa it is possible to determine the stage of the oestrous cycle.]

(*b*) **Gestation.** During gestation changes appear gradually in the wall. The intermuscular connective tissue is increased and becomes looser: this separates the muscle fibres from one another and these fibres also enlarge. The epithelium is thickened and the blood vessels, especially the veins, enlarge.

Blood Vessels and Lymphatics

Large arteries are found in the outer part of the mucous coat and the issuing veins make plexuses in the muscle coat. The lymphatics follow the distribution of the blood vessels.

Nerves

Free sensory nerve endings are present among the epithelial cells. Non-myelinated fibres supply the muscle coat and the blood vessels. Small and scattered autonomic ganglia are found on the course of the nerves.

THE EXTERNAL SEXUAL ORGANS

Clitoris

The clitoris corresponds to the penis of the male, but is much smaller and is not traversed by the urethra. It consists of two small masses of erectile tissue covered by the mucous membrane of the vestibule: this mucous membrane contains many sensory nerve fibres with specialized endings.

Labia

The *labia minora* consist of a core of loose and very vascular connective tissue, covered with stratified epithelium. There are many large sebaceous glands but no hairs.

The *labia majora* are folds of skin, the outer surface only being provided with hairs. The fold contains adipose tissue and some smooth muscle. There are many sweat glands and sebaceous glands opening on to both surfaces.

The connective tissue of the labia contains many specialized sensory nerve endings.

THE MAMMARY GLAND

The mammary glands are two, large, compound racemose glands in the superficial fascia that undergo characteristic changes at different periods of life.

Each gland consists of fifteen to twenty *lobes* radiating towards the *nipple*. The lobes are separated from one another by connective and adipose tissue and each lobe is provided with a *lactiferous duct* which emerges at the *nipple*. Surrounding the nipple is a pigmented area of skin, the *areola*, and beneath the areola each duct has a small dilatation or *ampulla* before narrowing down to open on the surface. Each lobe is divided into *lobules* by dense interlobular

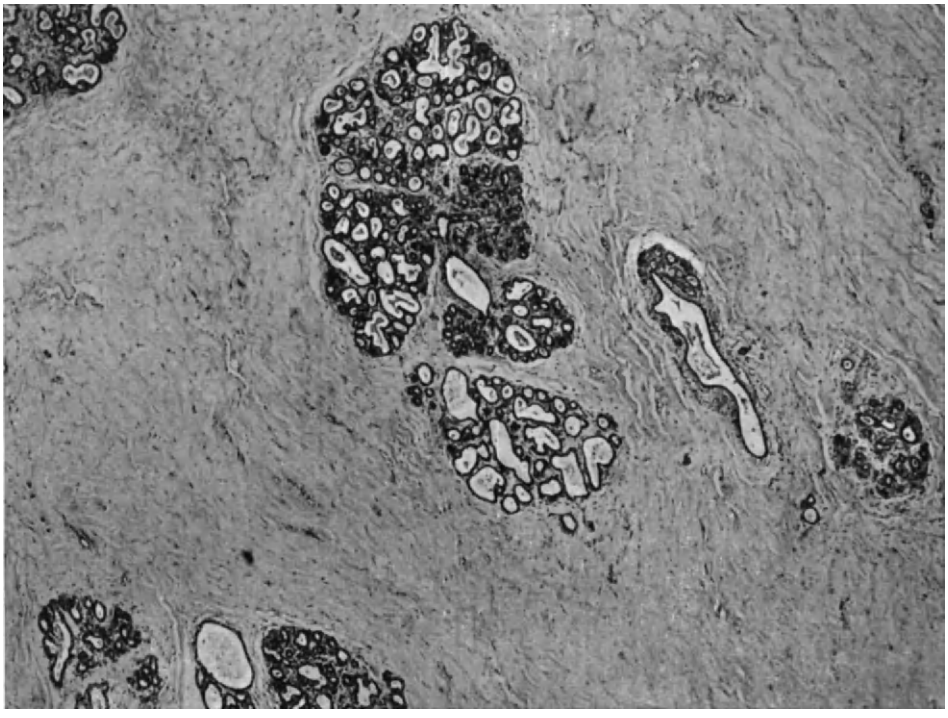

FIG. 24.22. A section of the mammary gland in early pregnancy. The lobules with their secretory alveoli may be seen, separated by interlobular connective tissue. 150 μm.

connective and adipose tissue (Fig. 24.22): the lobules contain the secreting *alveoli* and their *ducts*, together enclosed in specialized, cellular, periductal (or intralobular) connective tissue. This loose, vascular connective tissue contains little fat but many cells, thus allowing for easy distension when the alveoli multiply and enlarge for lactation.

The alveoli are lined by a layer of low columnar cells: external to these is a layer of elongated myo-epithelial cells resembling smooth muscle, and outside this again a basal lamina. The ducts are lined by two layers of cubical cells: the main ducts, near their surface opening, acquire a stratified epithelium.

The Nipple and Areola

The skin covering this area is thin and has well-marked papillae; the epidermis is pigmented, especially after pregnancy has occurred. The connective tissue contains smooth muscle bundles, arranged both along the ducts and circularly round the papillae. There is also much elastic tissue, and cords of white fibrous tissue, and the whole structure is so arranged that erection of the nipple can occur. In the periphery of the areola there are large sweat glands and sebaceous glands unconnected with hairs.

Physiological Variations in Structure

(1) The Gland before Puberty

The glands arise as solid epithelial buds growing down from the skin: these canalize before birth, and by continued growth and division a series of branching ducts arises which are embedded in the connective tissue. No alveoli are formed before puberty. In the male the glands develop at first as in the female, but after about eleven years they undergo involution. In the female the glands continue to develop slowly, and with the onset of sexual maturity the rate of development increases greatly and alveoli are formed. The development and the activity of the gland are under the control of the hormones of the ovary, which are controlled in turn by the hormones of the adenohypophysis.

(2) The Resting Adult Gland

This is the state of the glands after puberty up to pregnancy and between successive periods of lactation. The bulk of the gland consists of connective tissue and adipose tissue, with a few scattered groups of ducts and terminal alveoli; the latter are lined by a low cubical epithelium. At every sexual cycle there is a mild degree of growth and activity followed by regression.

(3) The Gland during Gestation

During the first part of pregnancy the epithelium of the terminal parts of the ducts multiplies actively and secretory alveoli are formed. The branching terminal ducts and alveoli are arranged in groups (lobules) closely packed round with the very cellular periductal connective tissue. As the glandular tissue increases this connective tissue becomes reduced to vascular packing between the alveoli. This growth necessitates the disappearance of much of the interlobular adipose and connective tissue: infiltration with lymphocytes is common. During the latter part of pregnancy the cellular proliferation ceases and the cells enlarge and begin to produce a secretion. At the end of pregnancy the *colostrum* is produced. This differs from the true milk partly because it contains very little fat, but also because it contains many cells and fragmentary cells: these cells are probably in part detached glandular cells and in part migrated lymphocytes, containing fat globules: they are known as colostrum corpuscles.

(4) **The Gland during Lactation** (Fig. 24.23)

When the mammary gland is actively secreting, different alveoli show different stages of activity. If the alveolus or duct is full of milk the lumen is wide and the wall thin: if little secretion is in the lumen the wall is thick and the lumen narrow: in some parts the cells are full of secretion and in others they are empty. Thus the secretory cells vary in shape from flat to columnar.

The secretory activity of the cells is shown by the presence of droplets of fat that accumulate towards the free surface of the cells: this fat appears as vacuoles in a paraffin section. The secretory cells show with the E/M much rough surfaced endoplasmic reticulum and a prominent Golgi apparatus. The rough endoplasmic reticulum probably elaborates the protein compo-

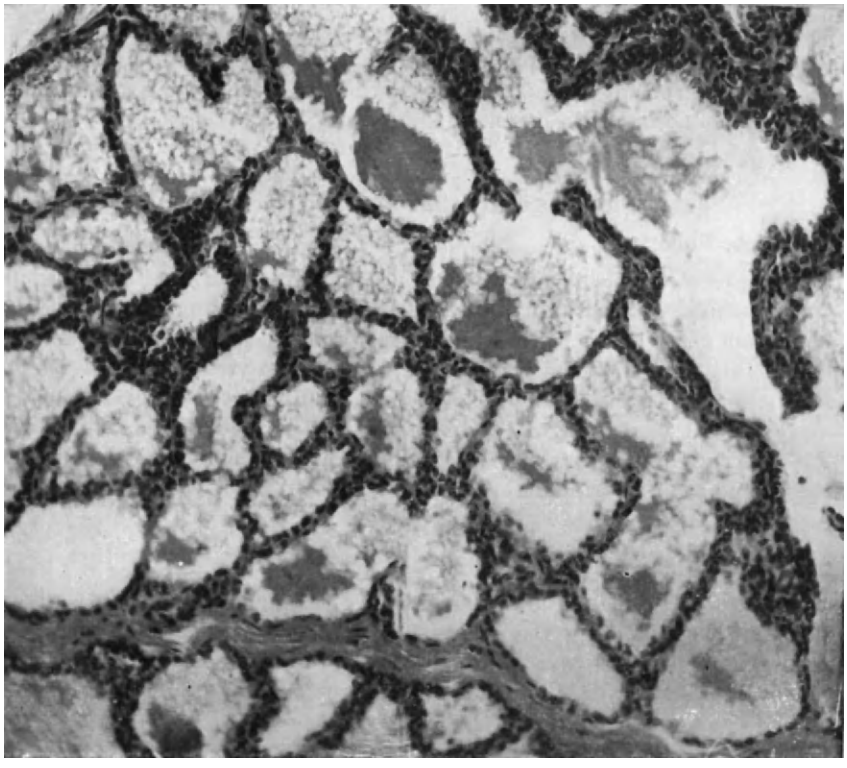

FIG. 24.23. High power photomicrograph of an actively secreting mammary gland during lactation. The alveoli have coagulated protein within their lumens. 10 μm.

nents of the milk; these appear as electron-dense granules in the vesicles of the Golgi complex. From here they pass to the apex of the cell and are released by the fusion of their membranes with the plasma membrane of the cell surface. The lipid droplets which are such a characteristic feature of the milk seem to arise in the cytoplasmic matrix; they move towards the apical cytoplasm of the cell where they accumulate and bulge out into the lumen of the alveolus. They remain covered by a thin rim of the apical cytoplasm of the cell. This eventually breaks off, so liberating the lipid droplets into the lumen of the secretory alveolus. The cells quickly accumulate more secretion and the process is repeated.

The terminal ducts are lined by cubical or columnar epithelium, with spindle-shaped myo-

epithelial cells between them and the basement membrane: all but the terminal ducts have a lining of two layers of epithelium.

The connective tissue between the lobules is much less than that of a resting gland: near the nipple the interstitial tissue contains smooth muscle. The periductal (interalveolar) tissue is loose and extremely vascular: it contains many cells of which fibroblasts, macrophages, lymphocytes and plasma cells are the most numerous. (The presence of granular leucocytes indicates an inflammatory process.)

(5) The Mammary Gland after Lactation

At the end of the nursing period the gland returns to its resting condition. Any secretion remaining is absorbed, the alveoli shrink, and most of them disappear, and there is an increase in the connective tissue.

(6) The Mammary Gland after the Menopause

In post-reproductive life the gland undergoes involution and returns to a condition similar to that before puberty: the process is slow and intermittent. The connective tissue becomes less cellular and less distinctly fibrillar, and fat is deposited: the whole mass appears more or less homogeneous, with small islands of inactive ducts and alveoli. At this time pathological processes are of fairly frequent occurrence in the epithelial cells.

Blood Vessels

The blood vessels ramify in the interlobular connective tissue, and supply capillary networks for the alveoli and the ducts. The blood supply is much increased during activity of the gland.

Lymphatics

The lymph capillaries make networks round the alveoli and ducts, and lead into larger lymphatics in the connective tissue draining toward the subcutaneous lymph plexus: from here they ultimately drain chiefly to the lymph nodes of the axilla.

Nerves

Nerve fibres supply the blood vessels and the muscular elements in the alveolar and duct walls. Delicate free nerve endings have been described in the mammary gland between the epithelial cells, and Pacinian corpuscles are found among the ducts. The nipple has a rich innervation, and large numbers of many types of sensory somatic nerve endings are present.

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CHAPTER 25

THE EYE

The organ of vision consists of the eyeball with its optic nerve: associated with the eyeball are the eyelids and the lacrimal apparatus.

THE EYEBALL (Fig. 25.1)

The eyeball is almost spherical and consists of a wall enclosing a cavity that contains the vitreous and the aqueous humours, with the crystalline lens suspended between them. The wall consists of three coats: (1) the external fibrous coat, comprising the *cornea* and the *sclera*; (2) the middle vascular coat (sometimes called the *uvea*, *uveal tract* or *tunica vasculosa*), consisting of the *choroid*, the *ciliary body* and the *iris*; (3) the internal nervous coat, or the *retina*.

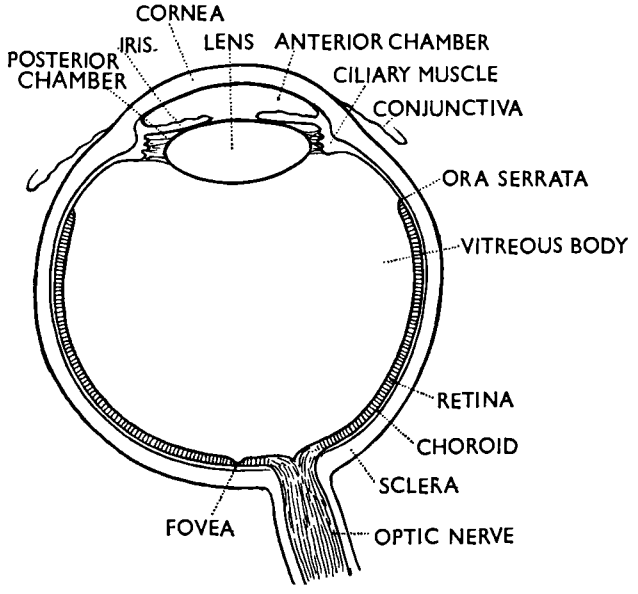

FIG. 25.1. A diagram of the eyeball in horizontal section to show the general structure.

Conjunctiva

This is the name given to the mucosal layer which covers the anterior part of the eyeball (the *bulbar conjunctiva*) and the posterior part of the eyelid (the *palpebral conjunctiva*). It is a stratified cuboidal epithelium 4–5 cells thick which is unusual in that in the palpebral region it is well provided with goblet cells. The conjunctiva is continuous with the epithelium of the cornea and at the junctional region (the *limbus*) the conjunctival epithelium becomes stratified squamous and thickens to form a layer about ten cells deep. The conjunctiva is often the site of inflammations, when the blood vessels in its lamina propria show considerable swelling.

Sclera (the sclerotic coat)

The sclera forms the posterior fourfifths of the eyeball and is continuous anteriorly with the cornea. It has a thickness of about 0·5 mm. The sclera consists of dense fibrous tissue

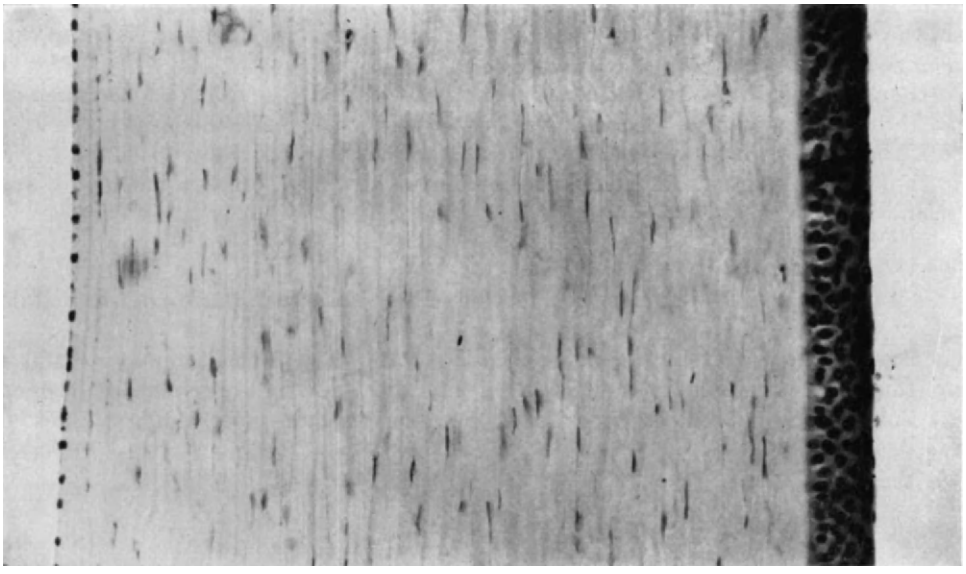

FIG. 25.2A. Photomicrograph of the cornea in section; micrograph by Dr. A. Lockett. 70 μm.

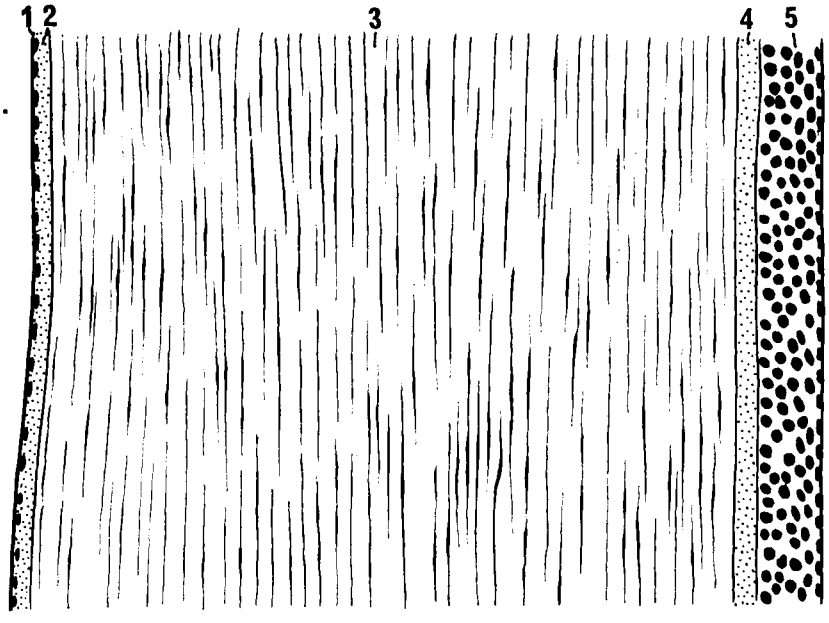

FIG. 25.2B. A diagram of Fig. 25.2A to show the layers: 1, inner layer of squamous epithelium; 2, Descemet's membrane; 3, stroma or substantia propria; 4, Bowman's membrane; 5, external stratified epithelium.

arranged in a rather irregular fashion, unlike that in the cornea; there is also much more elastic tissue than in the cornea. The tendons of the extrinsic eye muscles are continued into the sclera in order to provide rotation of the eyeball. Externally many of the collagen bundles are continued into a very loose layer containing clefts filled with lymph, the *episcleral space*: this is surrounded by dense connective tissue of the fascial sheath of the eyeball (*capsule of*

Tenon). The cells of the sclera are few in number, and those near the corneal junction and the exit of the optic nerve contain pigment granules: the internal layer of the sclera also contains pigment cells, and is known as the *lamina fusca*.

At the point of exit of the optic nerve the sclera is reduced and forms a fenestrated *lamina cribosa* through the spaces of which the various nerve fibres forming the optic nerve leave the eyeball. The lamina cribrosa constitutes a weak spot in the wall of the eyeball, and any rise in the intra-ocular pressure (as for example in the disease *glaucoma*) causes a bulging outwards of the scleral fibres at this point.

Cornea (Fig. 25.2)

This is the transparent anterior continuation of the sclera, and consists of five well-defined layers.

(1) External stratified epithelium, continuous with that of the conjunctiva and about 50 μm. thick. These cells are characterized by their great powers of regeneration if damaged.

(2) Bowman's membrane, a highly developed homogeneous layer 5–10 μm. thick.

FIG. 25.3. A spread preparation of the cornea treated with a metallic salt to show the keratocytes. Photograph by Dr. A. Lockett. 10 μm.

(3) Stroma or substantia propria, constituting the main mass of the cornea. It is continuous with the sclera and consists of very regularly arranged lamellae of collagen fibres. In each layer the collagen fibres are parallel with one another, but in adjacent layers the fibres change direction and are roughly at right angles to the fibres in the next layer. The layers also exchange fibres and the whole is held together by a mucoprotein which is highly sulphated and rich in keratan sulphate. As the fibres and the mucosubstance have the same refractive index and are highly hydrated, the whole cornea is transparent. Between the lamellae are very irregularly shaped flattened cells like fibroblasts; these are known as keratocytes (Fig. 25.3).

(4) Descemet's membrane (posterior lamina), a homogeneous layer about 5–10 μm. thick and probably very similar to an exceptionally well-developed basal lamina.

(5) Inner layer of squamous epithelium, 1 cell thick, continuous with the epithelium covering the anterior surface of the iris.

There are no blood vessels or lymphatics in the cornea; nutrition is slow and by diffusion through the intercellular matrix. The cornea is richly innervated with fibres which lose their myelin sheath on entering the cornea from the periphery. They provide a plexus in the substantia propria and another plexus beneath the epithelium that sends free nerve endings to terminate among the epithelial cells.

At the junction of the cornea and the sclera the collagen fibres are continuous and the basal lamina of the cornea is continued into the tissue of the irido-corneal angle (Fig. 25.4). Within

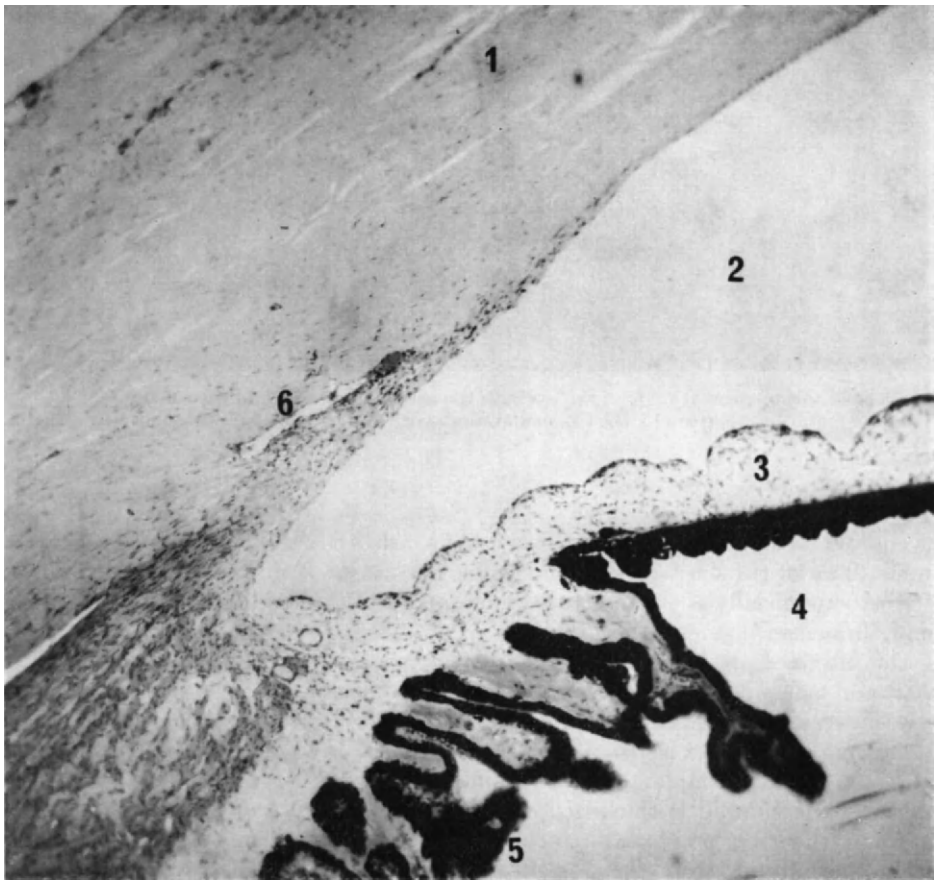

FIG. 25.4. A low power micrograph of the irido-corneal angle: 1, cornea; 2, anterior chamber; 3, iris; 4, posterior chamber; 5, ciliary processes; 6, canal of Schlemm. Micrograph by Dr. A. Lockett.

100 μm.

this tissue are spaces, the *trabecular meshwork* (Fig. 25.5), which are in communication with the anterior chamber of the eye and, like it, are lined by endothelium. They form the channels whereby fluid can pass from the anterior chamber into the circular venous sinus. This sinus is known as the *sinus venosus sclerae* or, more commonly, the *canal of Schlemm*; it drains into the anterior group of ciliary veins and usually contains only clear fluid. Obstruction of the trabecular meshwork causes the rise in the intra-ocular pressure which leads to the pathological changes of glaucoma.

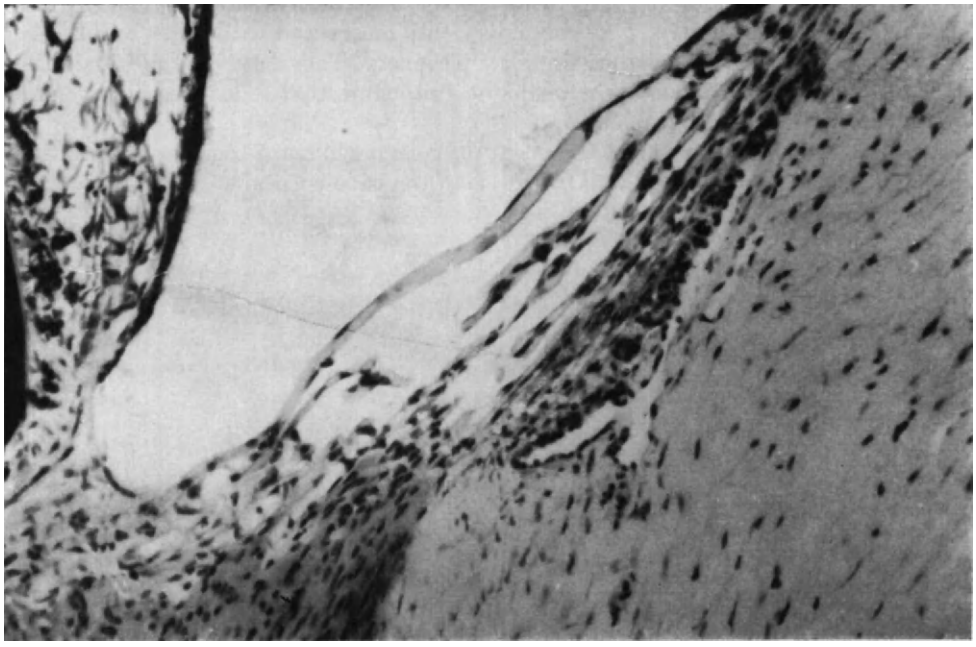

FIG. 25.5. The irido-corneal angle of the eyeball; the iris is on the left and the centre of the picture is occupied by the trabecular meshwork and the canal of Schlemm. 25 μm.

Choroid (Fig. 25.6)

The choroid is the vascular coat of the eye and consists of a thin brown layer separated from the lamina fusca of the sclera by lymph spaces. It consists of three layers:

(1) Most superficially is the *lamina suprachoroidea*, a thin membrane of connective tissue containing branched pigment cells.

(2) The intermediate layer is a vascular layer, containing large arteries and veins in its outer part and a network of extensive capillaries (the *choriocapillaris layer*) within (Fig. 25.17). Between the vessels is loose connective tissue; pigment cells are present in the outer layers.

(3) The innermost layer is *Bruch's membrane* (the *lamina vitrea*) a thin transparent membrane 1–4 μm. thick, resembling a basal lamina.

Anteriorly the choroid is thickened and forms the *ciliary body* which consists of *ciliary processes* and the *ciliary muscle*. Attached to the anterior edge of the ciliary body and separating the anterior from the posterior chamber of the eye is the *iris* which extends forwards as a diaphragm over the lens.

Ciliary Processes (Fig. 25.7)

These are about 70 radially arranged folds of the choroid, consisting of very vascular and pigmented connective tissue. They are covered by a vitreous membrane on which rest two layers of columnar cells, which constitute the forward continuation of the non-nervous part of the retina. Invaginations of the epithelium that appear to be small glands are present. The most interesting feature of the ciliary processes, however, is the presence in them of very large capillary networks. These have been shown by the E/M to have very thin endothelial walls. Perfusates of the blood plasma from these vessels enter the posterior chamber of the eye and form the *aqueous humour* which then circulates through the space between the front

of the lens and the posterior aspect of the iris to enter the anterior chamber of the eye. From here it drains back into the venous sinuses of the choroid via the trabecular meshwork and the canal of Schlemm.

Ciliary Muscle (Fig. 25.7)

This consists of smooth muscle, whose fibres are arranged in three groups: (*a*) an inner circular group near the base of the iris; (*b*) a middle radial group; (*c*) an outer meridional group.

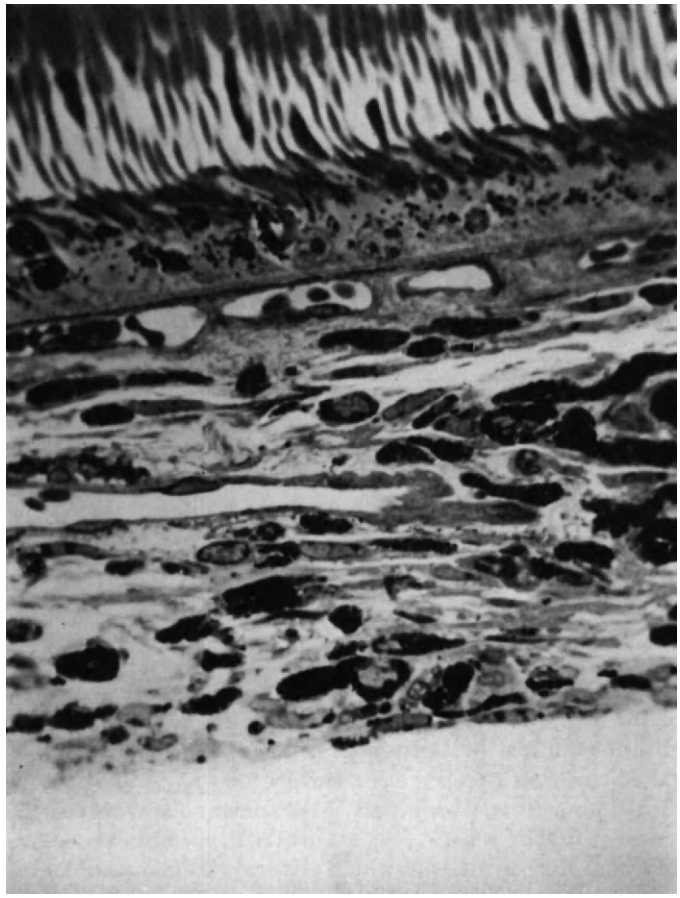

FIG. 25.6. A photomicrograph through a section of part of the retina and choroid. The preparation was embedded in Araldite and stained with toluidine blue. The outer segments of the retinal receptors are at the top of the picture with the pigment cell layer immediately below them. Bruch's membrane may be seen just above the very vascular choriocapillaris layer. Note the many pigment cells in the choroid connective tissue at the bottom; the sclera has been removed. Micrograph by Dr. A. Lockett.

8 μm.

These two last groups are sometimes called *Brücke's muscle.* In a section through this region this muscle appears as a triangular mass. The circular fibres are responsible for relaxing the tension on the lens and so allowing it to bulge by its own elasticity, thus accommodating the eye for near vision. There is a suggestion that the radial and meridional fibres may focus the lens for distant vision.

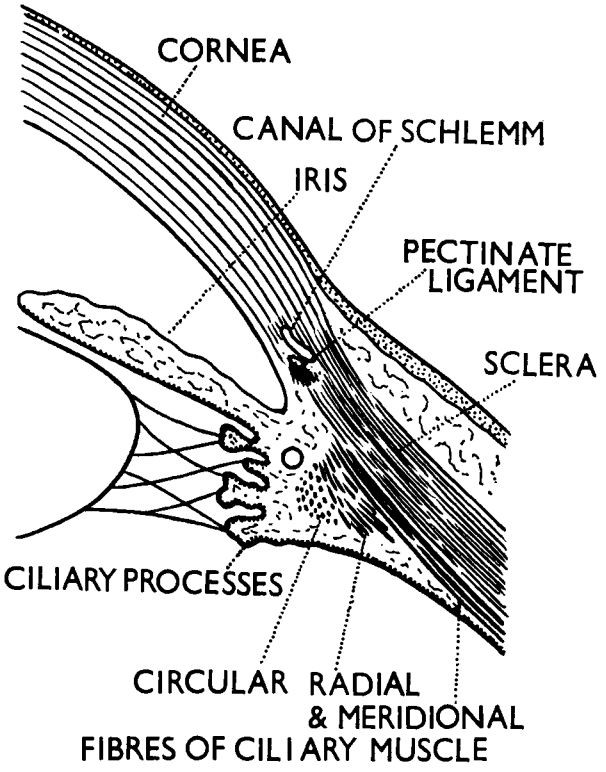

FIG. 25.7. A diagram of a meridional section of the eyeball to show the ciliary processes and the fibres making up the ciliary muscle.

The Iris (Fig. 25.8)

This is a further continuation forward of the choroid coat, and provides a circular adjustable diaphragm that projects into the eye with an aperture (the pupil) in its centre. It rests on the anterior border of the lens, dividing the anterior part of the eye into anterior and posterior chambers. The anterior surface of the iris is covered by a layer of squamous epithelium continuous with that covering the posterior surface of the cornea: the posterior surface is covered by an epithelium at least two layers thick continuous with that covering the ciliary processes and derived from the retina: it contains dark, melanin pigment. The substance of the iris consists of fine connective tissue which is very vascular and contains many pigment cells that determine the "colour of the eye" (Fig. 25.9). If this layer is thin and contains little pigment, the black pigment of the posterior surface as seen through it gives the iris a blue colour: more pigment produces shades of grey and green, while much dark pigment gives a brown iris. In albinos all the pigment is very scanty, and the red of the blood in the vessels combined with the colourless tissue produces a pink iris. In addition, the iris contains two groups of smooth muscle, the circular sphincter of the pupil near the pupillary margin, and the radiating dilator of the pupil lying more externally (Fig. 25.8).

The dilator pupillae muscle is supplied by post-ganglionic sympathetic fibres with their cell bodies in the superior cervical sympathetic ganglion; these fibres reach the eyeball along the long ciliary nerves. The constrictor pupillae fibres, on the other hand, have a parasympathetic innervation with the cell bodies lying in the ciliary ganglion and the post ganglionic fibres reaching the eyeball via the short ciliary nerves. These fibres also innervate the ciliary muscle

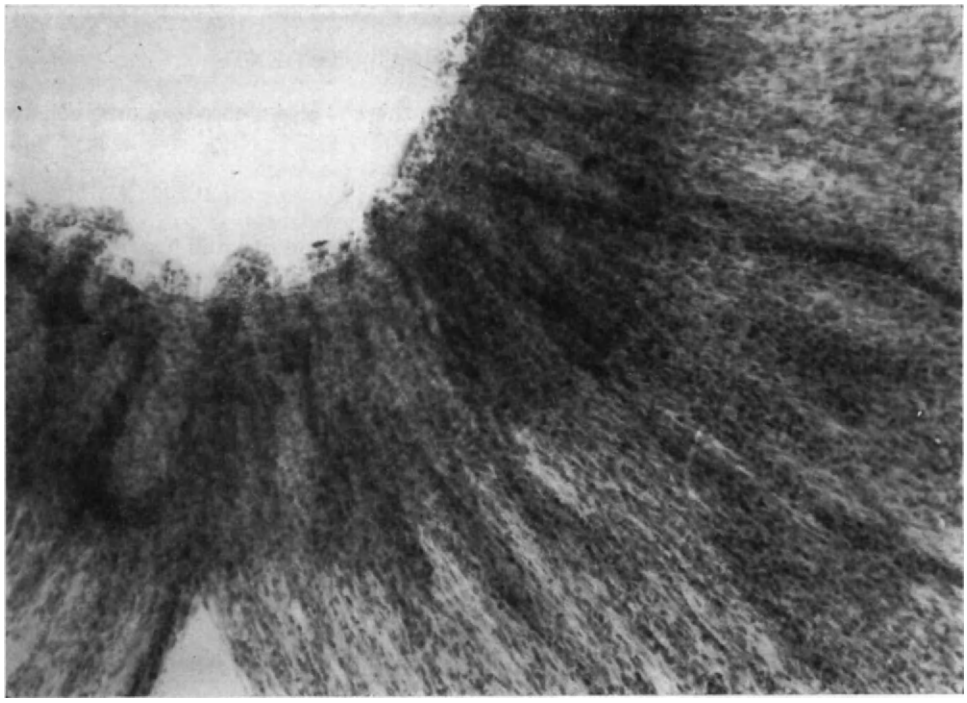

FIG. 25.8. Part of a whole mount of the iris, with the aperture of the pupil at the upper left. Note the darkly staining circular sphincter muscle and the radiating fibres of the dilator pupillae muscle. 75 μm.

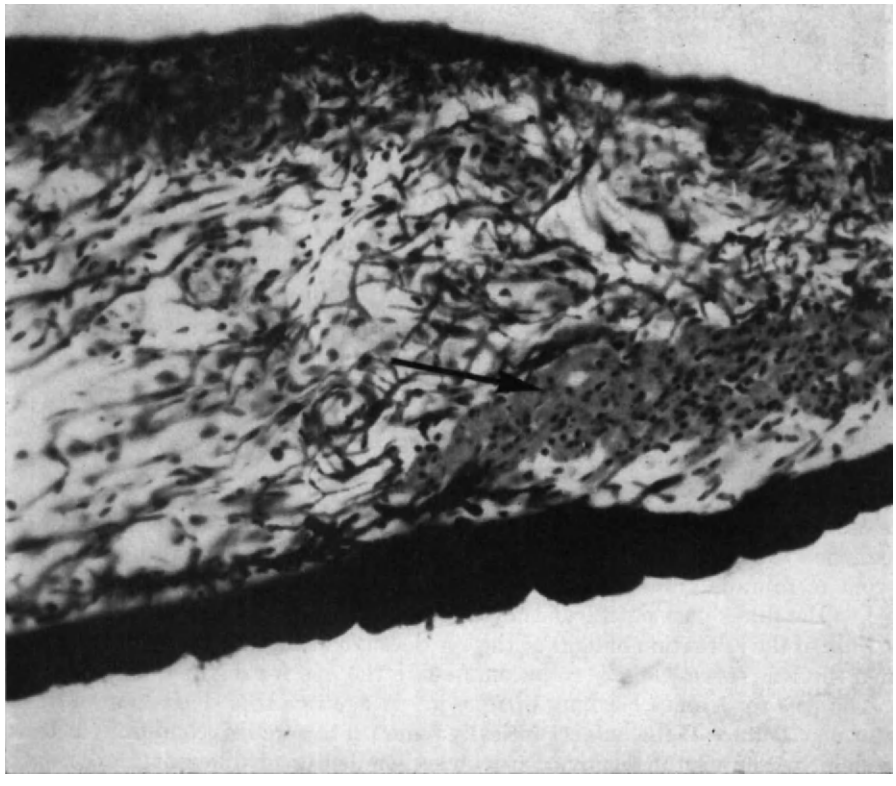

FIG. 25.9. A micrograph of part of the iris in section. The anterior surface is at the top of the picture and the pupillary margin at the right hand side. Note the very vascular pigmented connective tissue of the body of the iris and the deeply pigmented epithelium covering the posterior surface. The arrow indicates fibres of the sphincter pupillae muscle in transverse section. 35 μm.

so that when the eye accommodates for near vision there is also a corresponding constriction of the pupil.

The Lens

The lens is a transparent elastic body, biconvex in section and situated just behind the pupil. It is capable of small changes in shape which cause it to act as the means whereby fine focusing of the image thrown on the retina may take place. The lens is made up of fibrous laminae (Fig. 25.10) of very complex arrangement; the fibres are long hexagonal prisms held together by cement substance and having their nuclei near the middle. The lens is surrounded by an elastic capsule 8–10 μm. thick whose inner surface next to the lens substance is lined by a

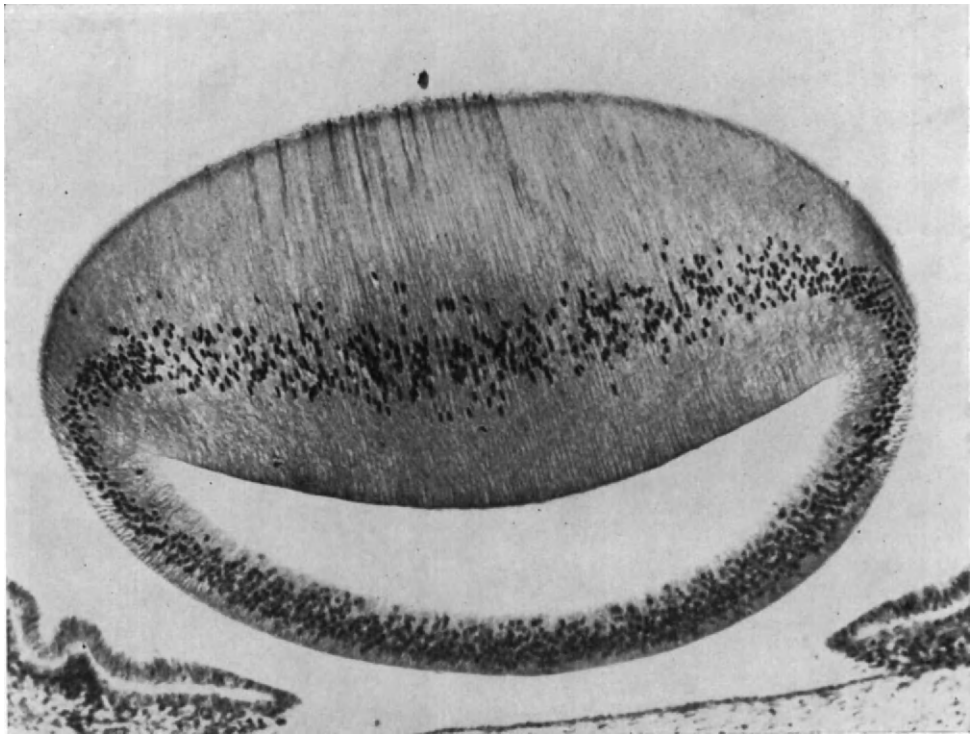

FIG. 25.10. A micrograph of the lens vesicle of a rat embryo. The fibrous lens prism cells are clearly seen; the cavity in the lens vesicle will subsequently close up. Photograph by Dr. G. N. Crawford.

125 μm.

layer of cubical or columnar cells. This layer of cells is absent from the posterior surface of the lens. Attached to the periphery of the lens capsule, both on its anterior and posterior surface is a fibrous membrane known as the *suspensory ligament* (the fibres are sometimes known as *zonules*); as the fibres pass outwards they are inserted into the ciliary region (Fig. 25.12).

The bulk of the refraction of light at the eye does not occur in the lens but at the corneal/air interface; the lens serves merely to accommodate the eye for distinct vision at varying distances. The lens sometimes becomes opaque in old age due to a deposition of calcium salts. This is termed *cataract*. If the lens is surgically removed to cure this condition, distinct vision is still possible by the use of biconvex spectacles but lenses of different focal lengths will be required for near and distant vision.

The Vitreous Body

The vitreous body occupies the bulk of the posterior part of the eyeball. It is a transparent gelatinous semi-fluid substance which contains fibres, connective tissue cells and some leucocytes. It is surrounded by the thin *hyaloid membrane*, which is continuous anteriorly with the suspensory ligament of the lens. Running through the vitreous from the centre of the lens to the point of entrance of the optic nerve is a canal, the *hyaloid canal*, which marks the site of the hyaloid artery in the embryo. This vessel is not found in the eye after birth.

The Retina

The retina lines the eyeball, ending at the margin of the pupil: its nervous elements extend only as far as the posterior limit of the ciliary body (to the ora serrata), the extension forward being entirely non-nervous. The nervous part of the retina is modified at the exit of the optic nerve and in the region of the macula. Its inner surface lies against the vitreous humour and its outer surface is adjacent to the choroid coat. The nervous part of the retina and the optic nerve are essentially parts of the brain. The retina of the living eye can be examined with the aid of an ophthalmoscope, an instrument which allows light to be projected into the eye through the pupil along the visual axis of the observer's eye. Under these conditions the retina is seen to have a red colour, due to reflection from the blood vessels in the underlying choroid, with a

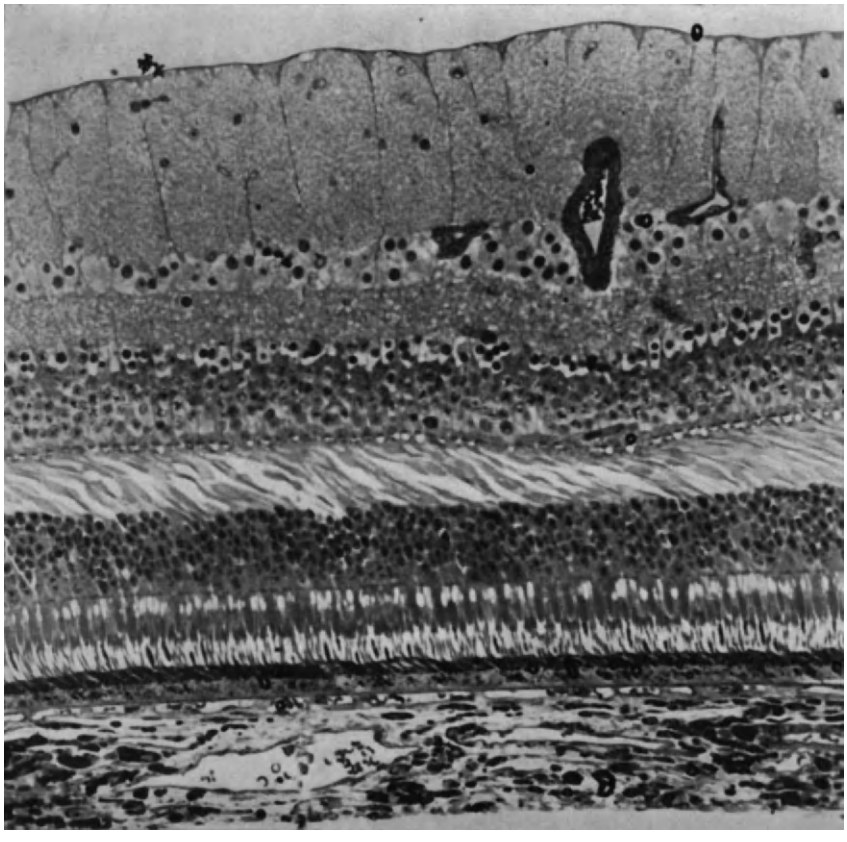

FIG. 25.11A. Photomicrograph of a section through the whole thickness of the retina and choroid. Micrograph by Dr. A. Lockett. 30 μm.

prominent yellowish area—the *macula*—which has a central depression known as the *fovea*. To the nasal side of the macula the *optic disc* may be seen. This is where the nerves leave the retina and where the blood vessels enter. These latter may be seen ramifying from the optic disc and spreading out over the inner surface of the retina. Ophthalmoscopy provides the clinician with a very valuable source of information.

The retina may be divided into several layers; from the outside these are (Figs. 25.11, 25.13):

(a) Pigment layer.
(b) The photoreceptor layer of rods and cones.
(c) External limiting membrane.
(d) External nuclear layer } These layers contain respectively the cell bodies and the
(e) External plexiform layer } synapses of the rods and cones.
(f) Internal nuclear layer (bipolar cells).
(g) Internal plexiform layer (synapses of the bipolar cells with the ganglion cells).
(h) Ganglion cell layer.
(i) Nerve fibre layer.
(j) Internal limiting membrane.

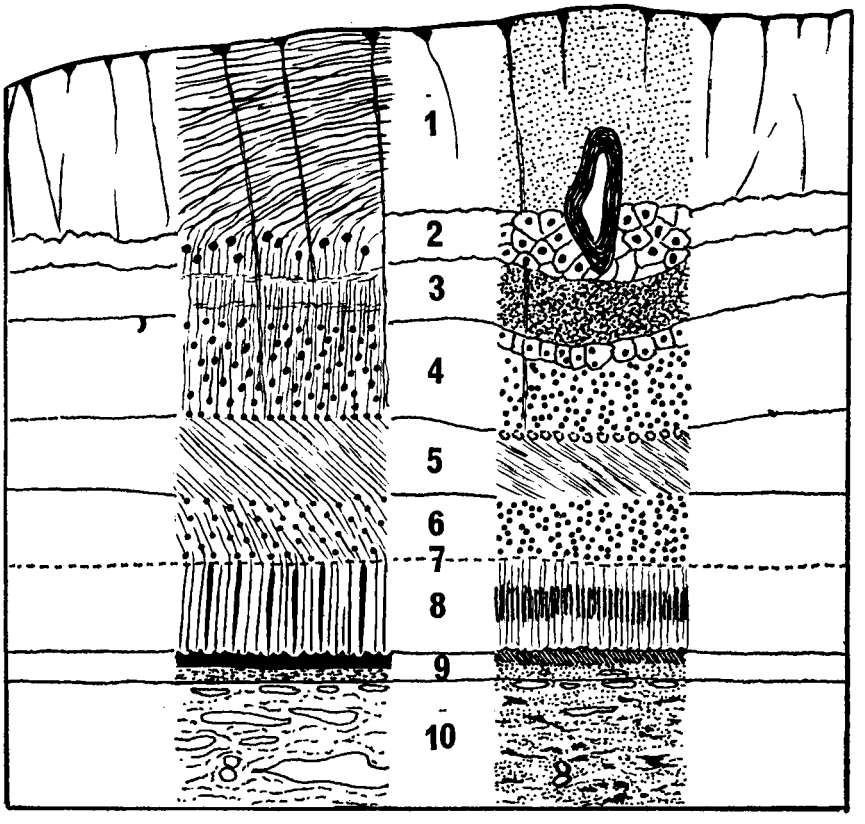

FIG. 25.11B. A diagram of Fig. 25.11A to identify the layers. 1, nerve fibre layer; 2, ganglion cell layer; 3, internal plexiform layer; 4, internal nuclear layer; 5, external plexiform layer; 6, external nuclear layer; 7, external limiting membrane; 8, photoreceptor cell layer—the outer and inner segments of the rods and cones; 9, pigment cell layer, separated by Bruch's membrane from 10, the choroid.

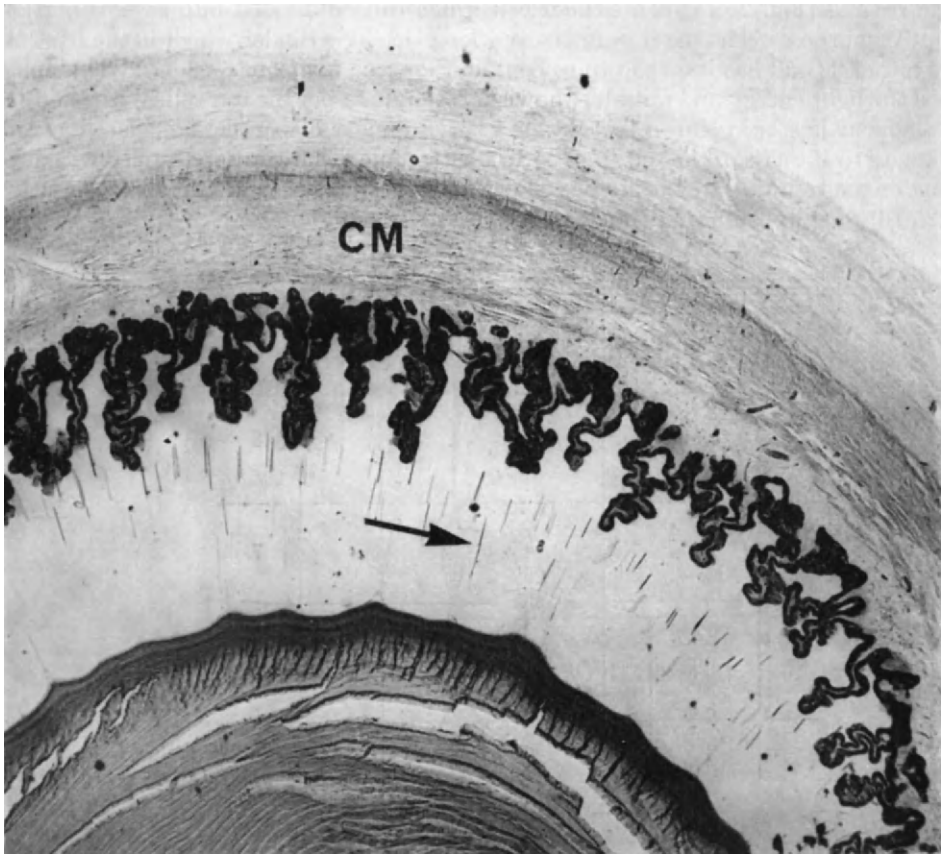

FIG. 25.12. Part of the lens of the eye and the adjacent tissues. The circular fibre of the ciliary muscle are marked CM; the ciliary processes with their pigment are clearly seen as are also the fibres of the suspensory ligaments of the lens (arrowed). 400 μm.

The *pigment layer* consists of large hexagonal epithelial cells resting against the lamina vitrea of the choroid and containing pigment: each pigment cell covers about nine or ten of the rods and cones. The cells send processes between these latter, and the processes contain pigment when the light entering the eye is bright.

The *photoreceptor layer* consists of cells of two kinds, *rods* and *cones*, which correspond to the first neurons in an afferent sensory receptor system. These cells have their axes arranged at right angles to the plane of the retina and because of the inversion which occurs during the embryological development of the eye, all the light which stimulates their photosensitive regions first has to pass through all the other layers of the retina. The rods far outnumber the cones in the human retina, one estimate being that there are between 110–125 million rods in the retina but only 6–7 million cones. As the total number of nerve fibres leaving the retina has been estimated to number only about 1 million it follows that there must be a large number of rods or cones connected via other intermediate neurons to any one fibre of the optic nerve. The rods are more numerous than the cones at the periphery of the retina but in the fovea there are only cones. In this region the cone density has been estimated at about 150,000 per square millimetre. The cones are used for colour vision and for the perception of fine detail whilst the rods are used for peripheral vision and for vision at low levels of light intensity.

The *rod cell* (Fig. 25.14) is a slender cell which can be divided into several portions for descriptive purposes. The outer segment is a long slender cylinder which in the fresh state is highly refractile and has been shown to contain the visual pigment *rhodopsin*. This substance absorbs the light energy and initiates the visual stimulus from the rod cell. With the E/M this outer segment has been shown to contain a large number of parallel lamellae orientated at right angles to the axis of the rod (Fig. 25.15). Ultrasonic disintegration coupled with negative staining of the resulting fragments has shown that these lamellae are, in fact, complete discs (Fig. 25.16) roughly 2 μm. in diameter.

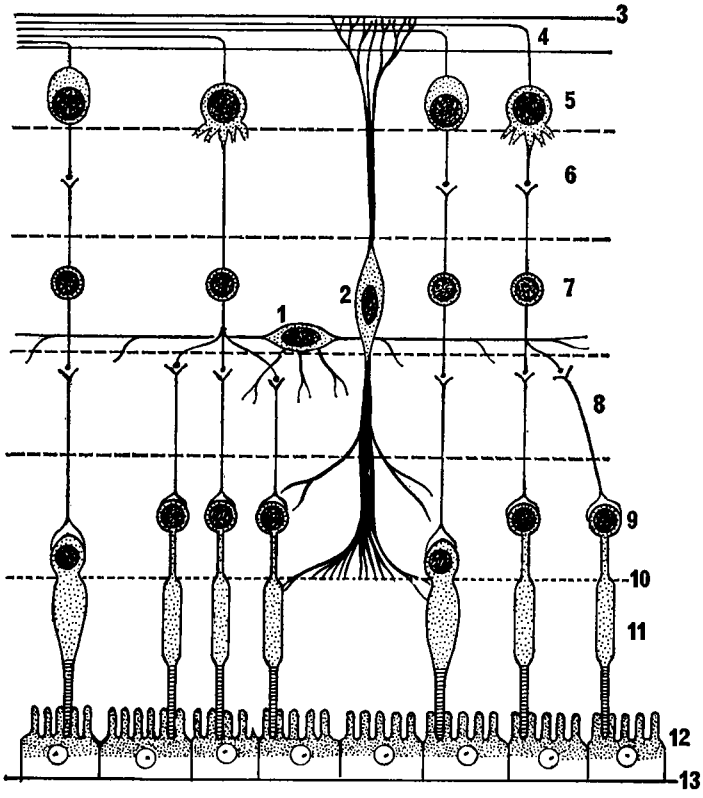

FIG. 25.13. A diagram of the retinal layers in simplified outline. 1, horizontal cell; 2, glial fibre (Müller's fibre); 3, internal limiting membrane; 4, nerve fibre layer; 5, ganglion cell layer; 6, internal plexiform layer; 7, bipolar cell layer; 8, external plexiform layer; 9, external nuclear layer containing the cell bodies of rods and cones; 10, external limiting membrane; 11, inner and outer segments of rods and cones; 12, pigment cells; 13, Bruch's membrane.

The outer segment of the rod is joined to the inner segment by a thin neck region (Fig. 25.15) which has been shown by the E/M to contain longitudinal axial filaments similar to those which normally are found in cilia; this has led to the suggestion that the rod and cone outer segments are, in fact, developed from modified cilia. The inner segment of the rod has a large number of mitochondria which are known as the *ellipsoid*. The cell bodies of the rods which contain their nuclei lie in the external nuclear layer, whilst the cellular process—the *rod fibre*—which is the homologue of the axon in a typical nerve extends into the external plexiform layer where they synapse with the bipolar cells (Fig. 25.13).

The *cones* (Fig. 25.14) have a very similar structure to that of the rods but their outer segment is much thicker and conical when seen in section, hence their name. The lamellate

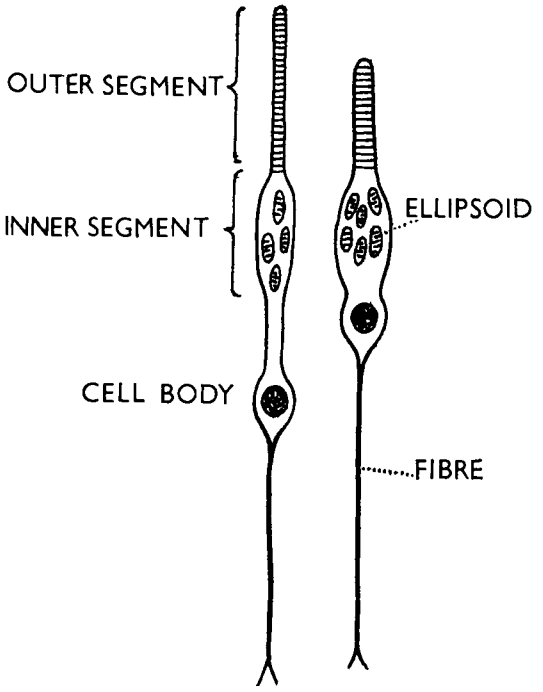

FIG. 25.14. A diagram of a retinal rod (on the left) and a cone to show their component parts. The aggregation of mitochondria in the inner segment is known as the ellipsoid.

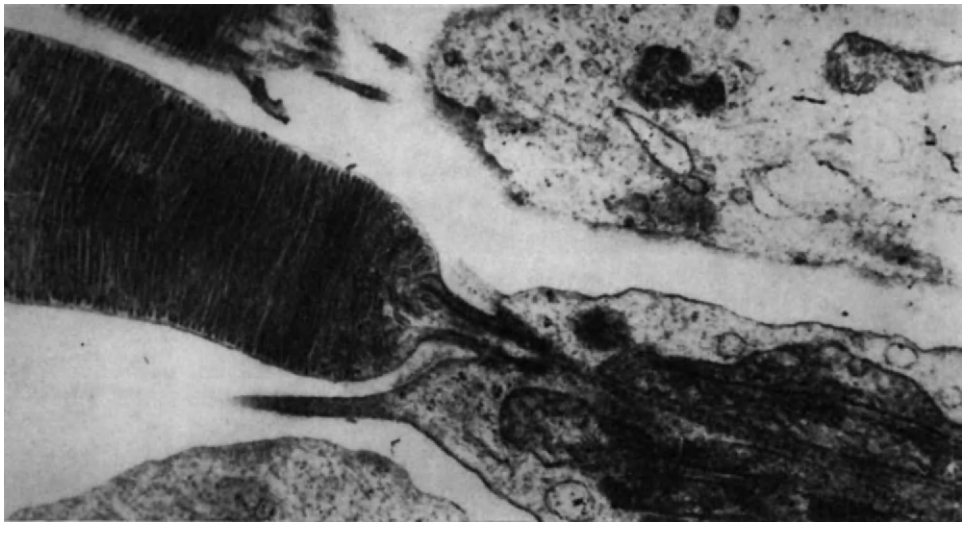

FIG. 25.15. An E/M of the outer segment of a rod cell in longitudinal section. The outer segment (on the left) shows the parallel lamellae; it is joined by a narrow neck to the inner segment. Some parts of the axial filament may be seen.

plates are also present but they do not contain rhodopsin but another visual pigment often called *iodopsin*. The cones are not identical all over the retina but are thinnest in the fovea where the visual acuity is greatest. As with the rods, the cone cell bodies are found in the external nuclear layer; their nuclei tend to lie immediately beneath the external limiting membrane and are, in general, larger and stain less intensely than those of the rods.

The rods and the cones are separated from one another by radially arranged glial fibres (Müller's fibres, Fig. 25.13) which extend through the bulk of the thickness of the retina. Lateral processes of these radial fibres contact the bases of the rods and cones and form a

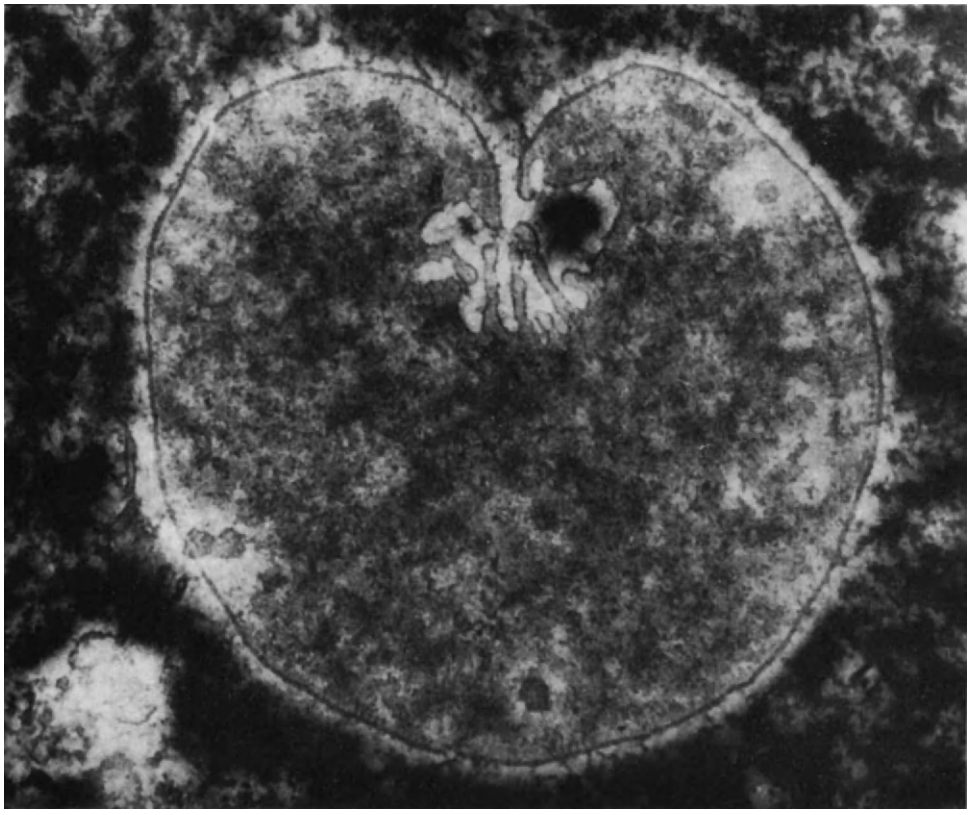

FIG. 25.16. An E/M of an isolated disc from the outer segment of a retinal rod. The preparation is negatively stained. Micrograph by Dr. C. M. H. Pedler. 0·2 μm.

series of desmosomal contacts with them which, when seen with the optical microscope, appear as a dense line known as the *external limiting membrane* (Fig. 25.17). The radial glial cells are very rich in glycogen.

The *external plexiform layer* is the region of synapse between the fibres of the rods and cones and the dendrites of the second neurons in the afferent chain. These latter cells are known as the *bipolar cells* (Fig. 25.13); they have their cell bodies and nuclei in the *internal nuclear layer*. This layer also contains the nuclei and cell bodies of a few large *horizontal cells* (sometimes called Müller's cells) which play an important role in the integration and summation of the responses. The bipolar cells synapse in turn with the large *ganglion cells*, the actual area of the synapse being in the *internal plexiform layer*. It seems that the bipolar cells are involved in the integration of the nervous impulses from the rods and cones before they are

transmitted to the ganglion cells which constitute the third order of afferent neurons. The ganglion cells are large neurons with prominent Nissl granules; their axons form the *nerve fibre layer* which ramifies over the surface of the retina before converging on the papilla to leave the eyeball as the optic nerve. As the retinal elements are absent at the papilla this is an area which is insensitive to light and is often known as the *blind spot*.

The *internal limiting membrane* forms the innermost layer of the retina and represents a basal lamina upon which the radial glial fibres ramify.

The nervous connection in the retina are immensely complex and they are only just being worked out. Most of the foveal cones appear to have a single line to the brain, via a single

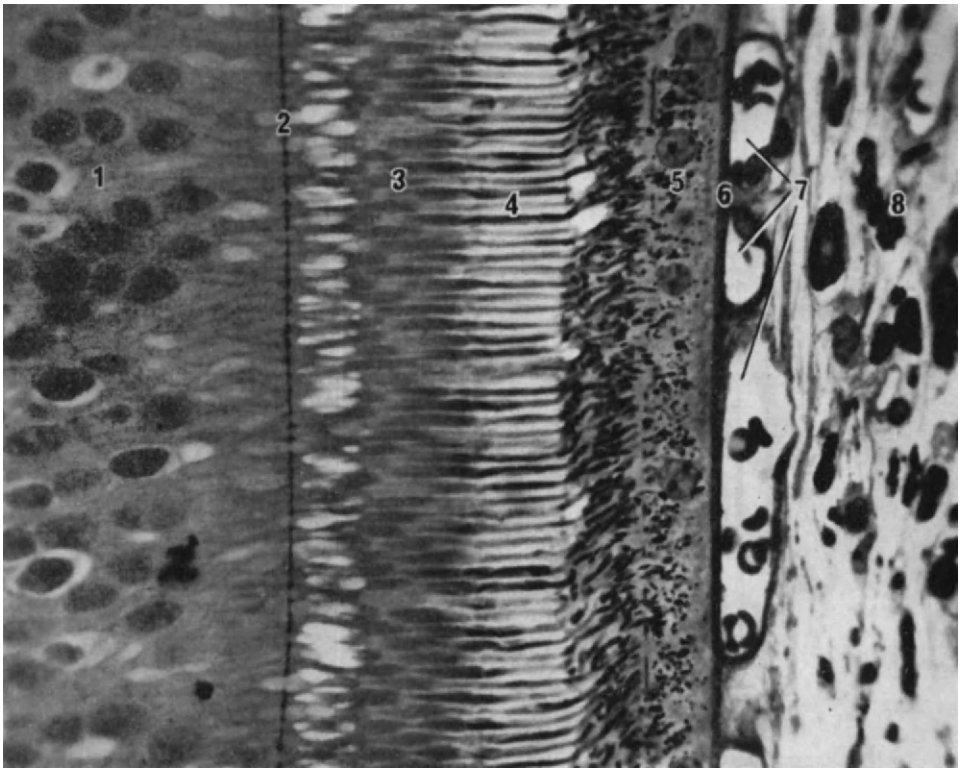

FIG. 25.17. An optical micrograph of part of a section through the retina and choroid. 15 μm. 1, external nuclear layer; 2, external limiting membrane; 3, inner segments of rods; 4, outer segments of rods; 5, pigment cell layer; 6, Bruch's membrane; 7, choriocapillaris layer; 8, choroid with pigment cells. Micrograph by Dr. A. Lockett.

bipolar cell and a ganglion cell (Fig. 25.18). In the case of rods at the periphery of the retina several are normally connected to one bipolar cell so that convergence exists. An extended stimulus may therefore act on several rods but only excite one nerve fibre. This would enhance the sensitivity of the system at low light levels. More convergence may take place as a result of the activity of the horizontal cells and of the connections at the ganglion cell level. Again, mixed populations of rods and cones may be linked to the same ganglion cell via one or more bipolar cells (Fig. 25.18).

In the *macula* and its central *fovea* the structure of the retina is modified for acuteness of vision. In the macula, an area 1·5 mm. in diameter, yellow pigment is found in most of the layers and the thickness (except at the fovea) is increased by an increase in the ganglion

cells and in the inner nuclear layer. The number of rods is much decreased, and in the fovea only cones are present. In the fovea the thickness of the retina is greatly reduced by an almost complete disappearance of all the layers other than the cones and the pigmented epithelium.

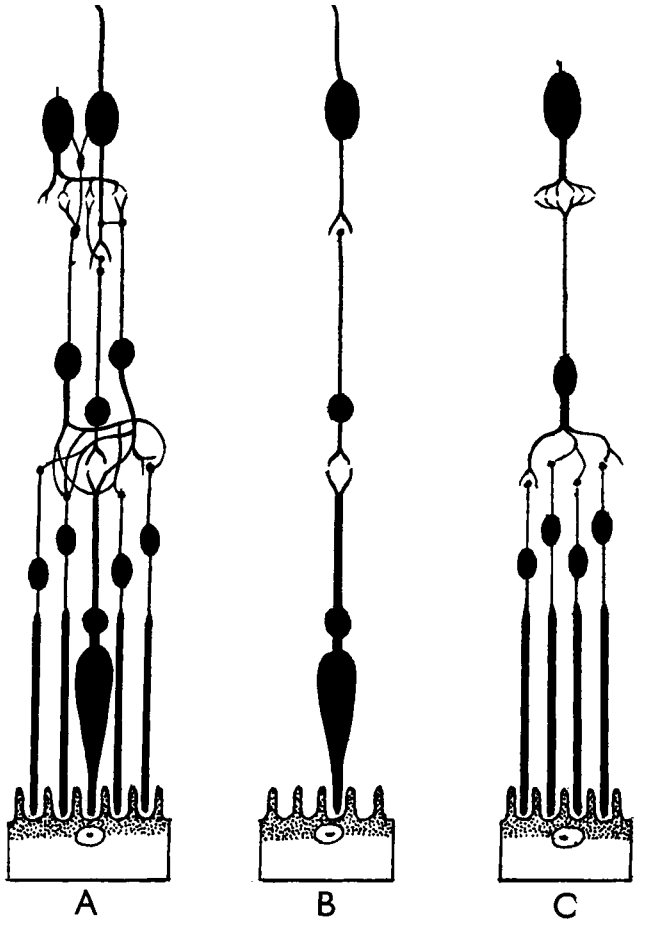

FIG. 25.18. Diagram of three possible connections between the rods and cones and the ganglion cells. A, 4 rods and 1 cone connect via 3 bipolar cells to 2 ganglion cells. B, 1 cone connects via a single bipolar cell to a single ganglion cell. C, 4 rods connect and summate via one bipolar cell to a single ganglion cell.

The Optic Nerve (Figs. 25.19, 25.20)

The fibres arising as axons of the cells in the ganglion layer of the retina are non-myelinated and pass in the layer of nerve fibres to converge on the point of exit of the optic nerve (the papilla or blind spot): these fibres in sweeping round avoid the fovea. Just after they leave the eye the fibres acquire a myelin sheath. The optic nerve (Fig. 25.19) is an outgrowth of the central nervous system and therefore the fibres have no neurilemma, and the whole is enclosed in the same sheaths as the brain itself. The outer sheath is the dura mater and is continued into the sclera. Beneath this is the subdural space and then the pia-arachnoid covering, consisting of two layers with a small subarachnoid space between them. (These spaces provide a path whereby infection may travel from the eyeball to the meningeal spaces of the brain.) The pia mater is closely adherent to the nerve and sends very fine trabeculae in among the nerve fibres,

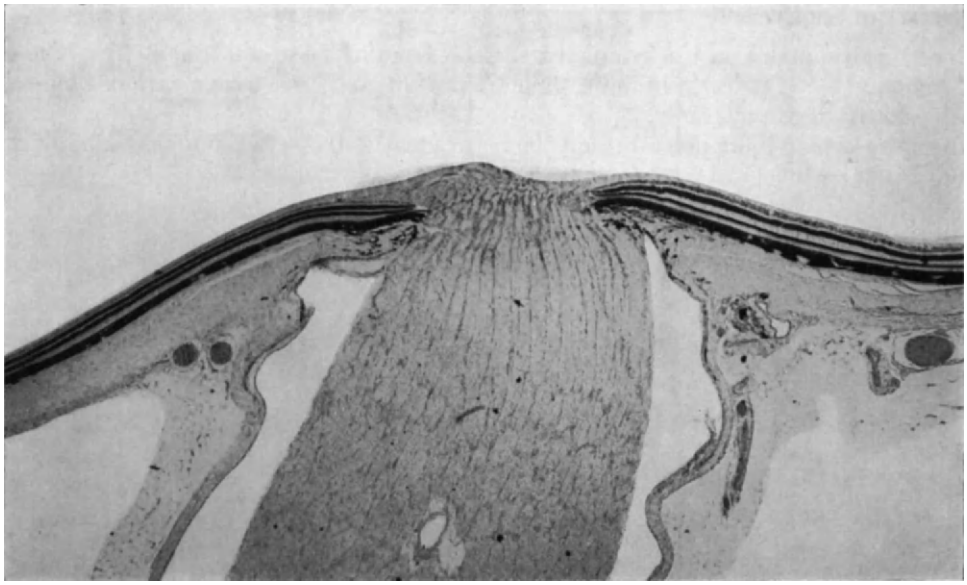

FIG. 25.19. A longitudinal section through the optic disc and nerve. 1 mm.

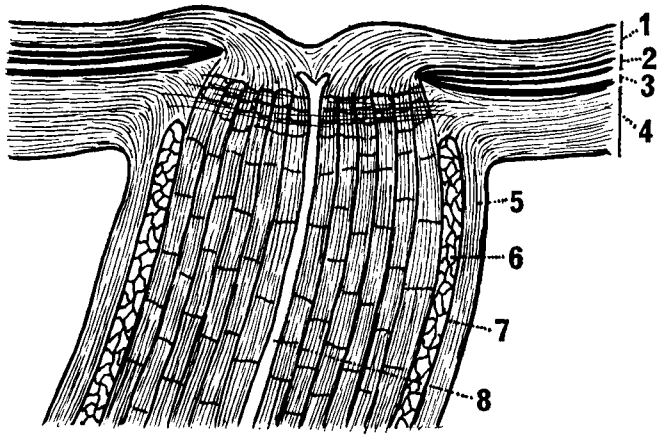

FIG. 25.20. A diagram of the structures in the region of the optic disc and nerve. 1, nerve fibre layer; 2, retinal receptor layer; 3, choroid; 4, sclera; 5, dural sheath of optic nerve; 6, subarachnoid space; 7, arachnoid; 8, central blood vessel (retinal artery).

which divide these into bundles and carry blood vessels. Neuroglia lies between the fibres and beneath the pial investment. For a short distance after leaving the eyeball the nerve carries the retinal artery and vein, lying in a strand of connective tissue (Fig. 25.21).

Blood Vessels of the Eyeball

The blood vessels can be divided into two independent groups; the *retinal* artery traverses the optic nerve and supplies the retina, whilst the *ciliary* group supplies the choroid together with the ciliary body, the iris, and the sclera. The central artery enters in the middle of the optic nerve and the vessels ramify in the nerve fibre layer: capillary networks extend to the inner nuclear layer, but the outer layers are supplied from the choriocapillaris layer of the choroid. The vein leaves in the optic nerve.

Lymphatics of the Eyeball

The eyeball contains no true lymphatic vessels except in the scleral conjunctiva. Numerous inter-communicating spaces containing fluid are present, and have been mentioned above, but they are not true lymph spaces.

The intra-ocular fluid pressure may increase greatly above the normal value of about 28 mm. Hg in pathological conditions (e.g. glaucoma).

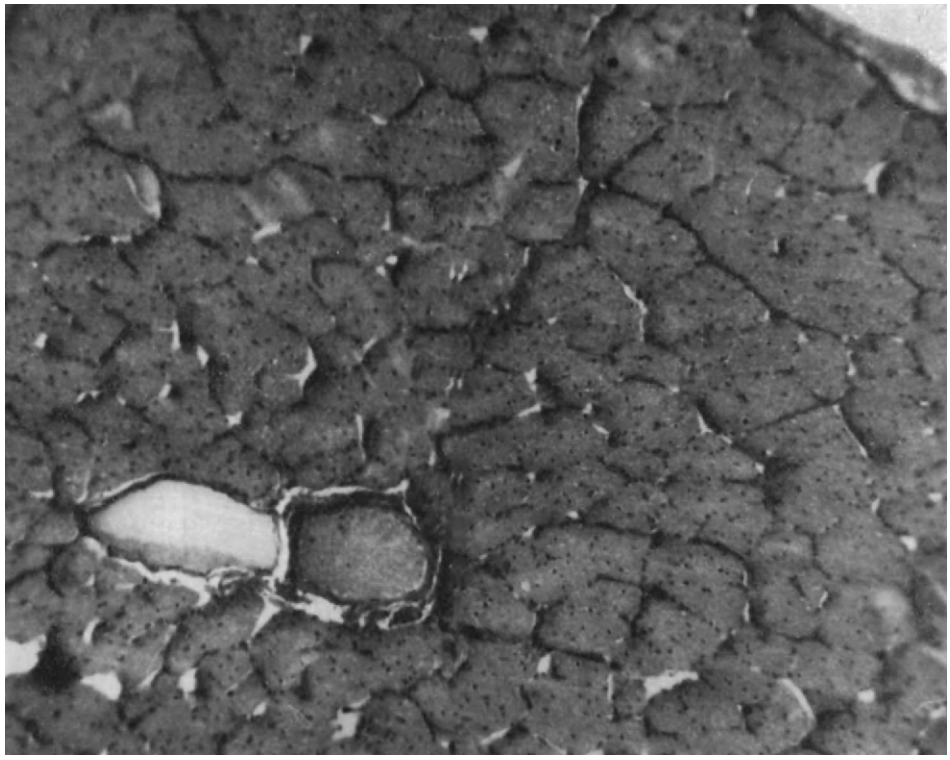

FIG. 25.21. A transverse section of the optic nerve to show the retinal artery and vein (at the lower left) in the centre of the nerve. 100 μm

Nerves of the Eyeball

The nerves of the eyeball include the *optic "nerve"* which is derived from the retina and is a tract of the central nervous system, and the *ciliary nerves* which supply the choroid coat, the ciliary body, the iris, and the sclera with sensory, sympathetic and parasympathetic fibres.

THE EYELIDS (Fig. 25.22)

The eyelid is covered externally by skin and lined internally by the conjunctiva reflected from over the anterior part of the eyeball.

The *skin* consists of a thin epidermis, with small dermal papillae. Sebaceous glands and small hairs are present and at the edge of the eyelid are large hair follicles for the eyelashes: the eyelashes are replaced every 100 to 150 days. Between the hair follicles, and opening into them, are large, coiled sweat glands, the *ciliary glands of Moll*: these are provided with large myo-epithelial cells.

The *middle layer* of the eyelid consists of connective tissue containing the thickening known as the tarsal plate which supports the eyelid, the Meibomian glands, and the striated palpebral part of the orbicularis oculi muscle. The numerous tarsal (Meibomian) glands are large lobulated sebaceous glands, with ducts opening externally on the margin of the lid behind the eye-lashes: their oily secretion helps to lubricate the edges of the eyelids so that they do not stick together, and also prevents the entry of water when the eyes are closed. Some smooth muscle attached to the tarsus is present in the upper eyelids.

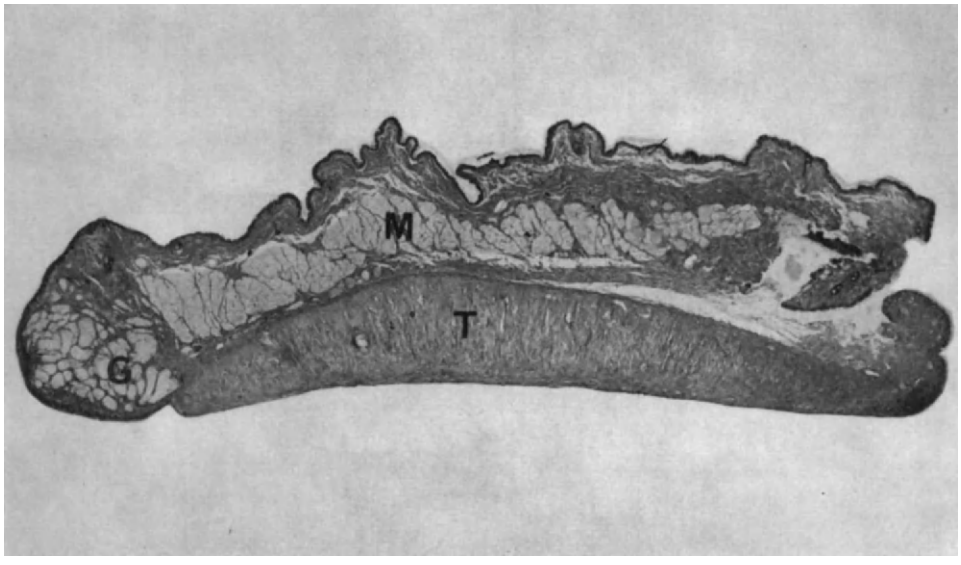

FIG. 25.22. A micrograph of a section through an eyelid. M, palpebral part of the orbicularis oculi muscle; G, Meibomian glands; T, tarsal plate. 1 mm.

Blood Vessels and Lymphatics

The arteries enter at each angle and unite to form the tarsal arch along the margin of the eyelid, and an external tarsal arch along the upper margin of the tarsus: capillary networks are given off from these arches. Lymphatic plexuses are present on each surface of the tarsus.

Nerves

The lid contains nerve plexuses from which fibres supply the glands, the blood vessels and the epithelium. Sensory end bulbs are present near the margin.

THE LACRIMAL APPARATUS

Each eye is provided with (1) a lacrimal gland and ducts supplying the eye with tears, and (2) two lacrimal canals, a lacrimal sac and a nasolacrimal duct conveying the tears to the nasal cavity.

The *lacrimal gland* (Fig. 25.23) is of the compound racemose type. The alveoli are lined by cubical serous cells separated in places from the basal lamina by well-defined myo-epithelial cells. Normally they are full of clear granules, but after copious secretion the cells appear empty. The interstitial tissue is very elastic. The smallest ducts are lined by a cubical epithelium, while in the larger ducts two layers of cells are present.

The gland opens by 6–12 ducts into the upper and lateral surfaces of the superior conjunctival fornix. The tears are washed across the conjunctiva and cornea by the blinking of the

eyelids and collect medially in the internal canthus of the orbit. From here the tears enter the *lacrimal punctae* (one on each eyelid) which represent the openings of the *lacrimal canals* which in turn are continuous with the nasolacrimal duct.

The lacrimal canals are lined by stratified squamous epithelium with a basal lamina and an elastic stroma containing diffuse lymphoid tissue. The lacrimal canals converge medially and

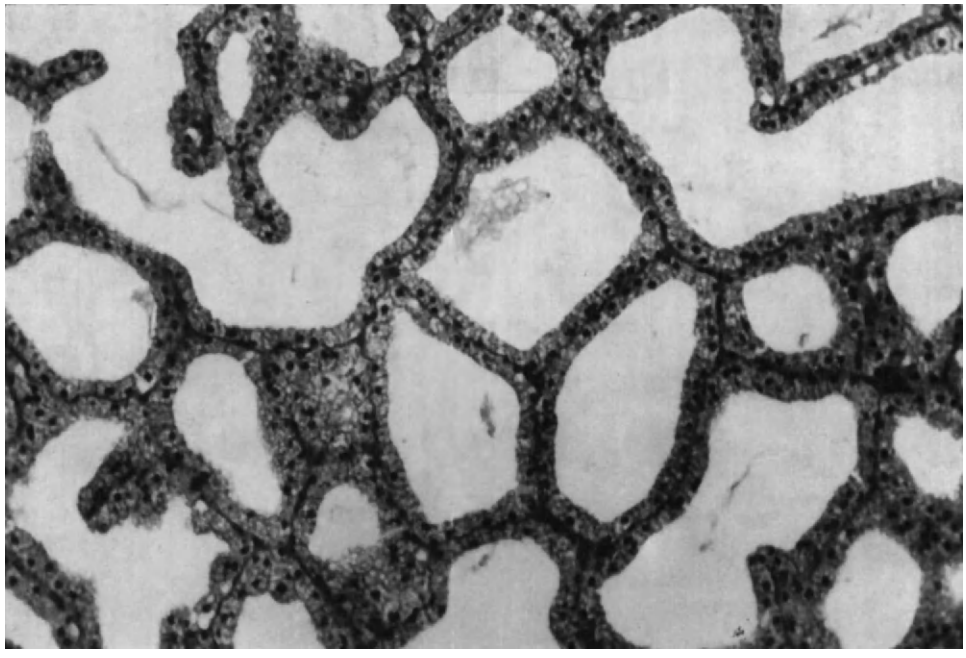

FIG. 25.23. A section through the lacrimal gland, showing the numerous alveoli each lined with cubical epithelium. 50 μm.

unite in the *lacrimal sac* which drains directly into the nasolacrimal duct. These have a lining similar to that of the canals but the epithelium is more columnar. The walls are separated from bony periosteum by a cavernous venous plexus.

The blood vessels, lymphatics and nerves are similar to those of other serous glands.

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CHAPTER 26

THE EAR

The ear (Fig. 26.1) consists of three parts: (1) the external ear (to receive the sound waves), (2) the middle ear (to transmit the vibrations), and (3) the inner ear (to receive the vibrations and originate the nerve impulses which are perceived by the C.N.S. as sound). The inner ear also is involved with equilibration and the perception of motion.

THE EXTERNAL EAR

The external ear consists of the *auricle* (pinna or concha), the *external auditory meatus* and the *tympanic membrane* (separating it from the middle ear).

The **auricle** is covered by a thin layer of skin containing hairs, large sebaceous glands and small sweat glands: in old men large stiff hairs often develop at the dorsal edge and lobe. The substance of the auricle consists of an irregular plate of elastic cartilage surrounded by perichondrium which contains many elastic fibres. A large number of fat cells are present in

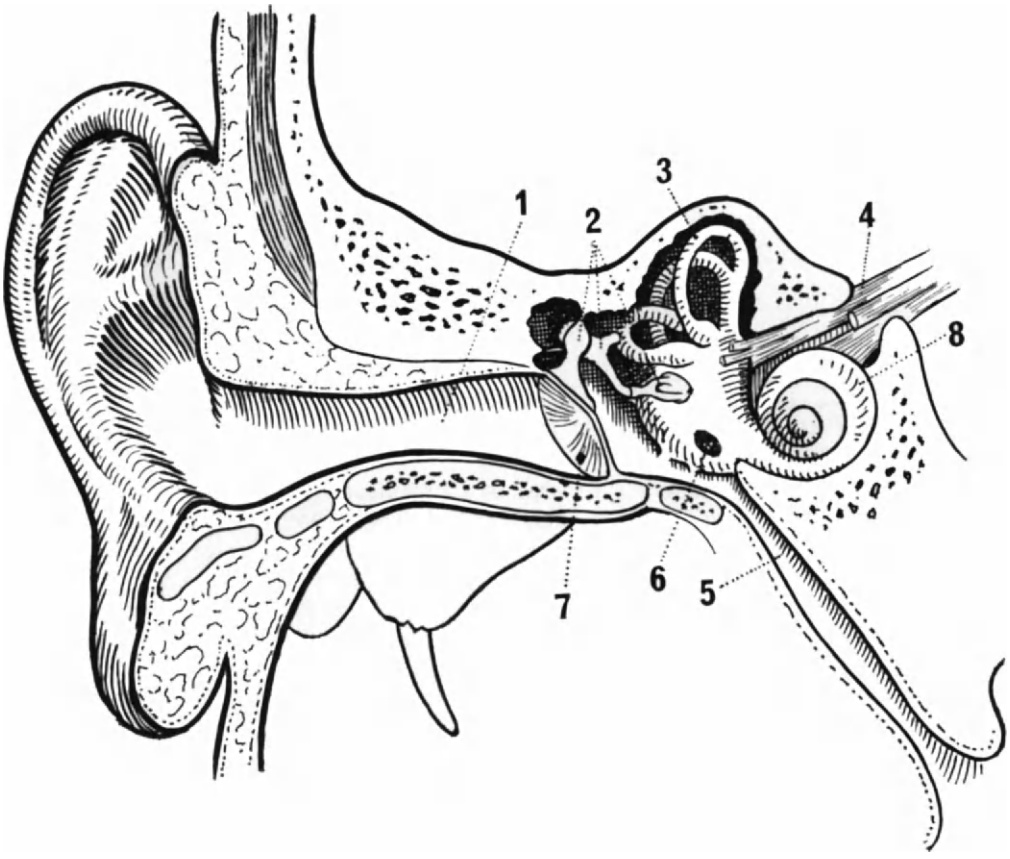

FIG. 26.1. A diagram of the organization of the parts of the ear. 1, external auditory meatus; 2, auditory ossicles; 3, semicircular canals; 4, auditory nerve; 5, pharyngotympanic tube; 6, fenestra rotundum; 7, tympanic membrane; 8, cochlea.

the subcutaneous tissue of the lower part of the lobe of the auricle. It is attached to the skull by small vestigial muscles.

The **external auditory meatus** is lined by a prolongation of the skin, which is reflected at the closed end over the surface of the tympanic membrane. In the outer part of the canal the skin is thick and contains protective hairs, sebaceous glands and large coiled ceruminous glands whose cells contain abundant lipid droplets. These glands are modified apocrine glands. Ear wax (*cerumen*) is composed of the secretion of the glands and of desquamated epithelium: it has a bitter taste, and protects the skin from drying, and also discourages the entry of insects. In the inner part of the canal the skin is thin and there are no hairs or glands. The wall is strengthened by cartilage in the outer part and by bone in the inner portion.

The **tympanic membrane** (Fig. 26.2) is a thin semi-transparent membrane separating the external ear from the middle ear (tympanic cavity): the handle of the malleus is attached to its inner surface. The outer side is covered by a very thin layer of skin continuous with that lining

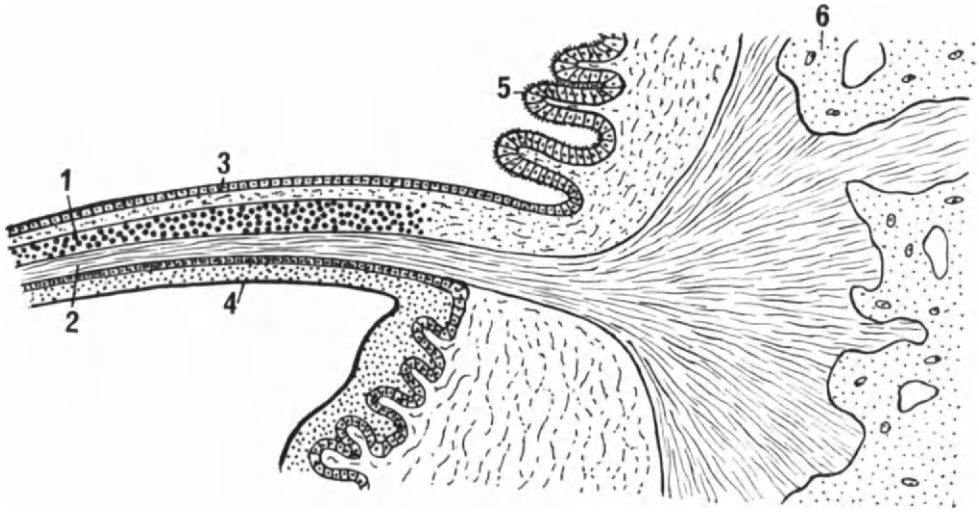

FIG. 26.2. A diagram of the tympanic membrane in section. 1, circular connective tissue fibres; 2, radial connective tissue fibres; 3, squamous epithelium; 4, thin layer of skin covering the outer aspect of tympanic membrane; 5, epithelium of tympanic cavity; 6, bone.

the external meatus. The inner side is covered by a squamous epithelium continuous with that lining the middle ear and overlying a stroma of connective tissue. This latter consists of close-packed collagen fibres arranged in two layers, separated by elastic networks.

The collagen fibres in the outer layer are arranged radially, extending from the point of attachment of the handle of the malleus at the umbo of the tympanic membrane to the periphery. The inner layer has its fibres arranged in a circular fashion. At the periphery of the tympanic membrane the fibres of the stroma form a fibrocartilaginous ring which is attached to the wall of the meatus.

Blood vessels, lymphatics and nerves are distributed for the greater part in the covering layers.

THE MIDDLE EAR (Fig. 26.3)

The middle ear consists of the tympanic cavity, an air-containing space in the petrous part of the temporal bone; it communicates with the nasopharynx by the pharyngotympanic (Eustachian or auditory) tube: it is bridged across by three ossicles. Posteriorly it communi-

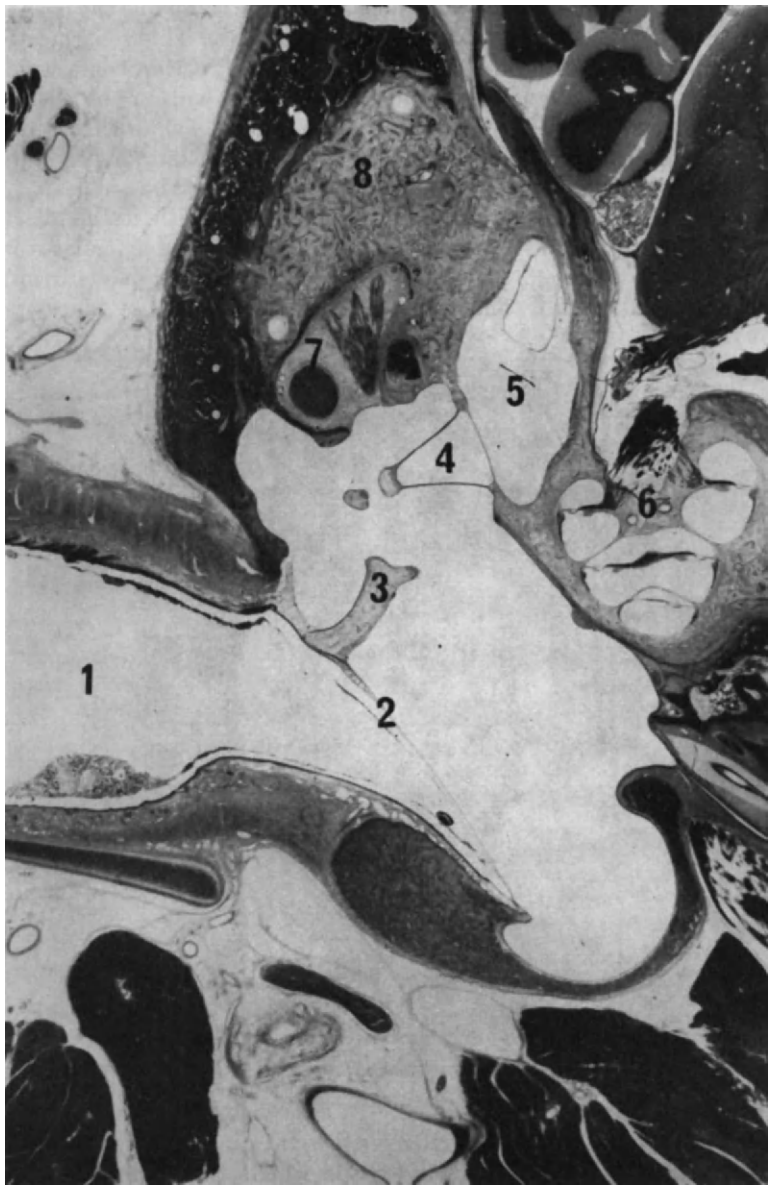

FIG. 26.3. A low power photograph of a section through the ear. 1, external auditory meatus; 2, tympanic membrane; 3, handle of malleus; 4, stapes; 5, semicircular canal in its bony tube; 6, cochlea; 7, facial nerve in its bony canal; 8, bone of the mastoid process.

cates through the tympanic antrum with the mastoid air cells of the temporal bone: this provides a path for a possible spread of infection from the middle ear.

The **tympanic cavity** is lined by a thin mucous membrane: the epithelium is for the greater extent of the squamous type, but near the opening of the pharyngo-tympanic tube it is columnar and ciliated. Beneath the epithelium is a thin stroma that contains some diffuse lymphoid tissue and is attached to the periosteum of the surrounding bone. This same lining is continued into the mastoid air cells which arise as diverticula from the tympanic antrum.

The medial wall of the tympanic cavity, which separates the middle ear from the inner ear, shows in its lower part the *round window* (*fenestra rotundum*). This separates the tympanic cavity from the scala tympani of the cochlea and is closed by a thin connective tissue membrane, covered on the tympanic side by mucous membrane and on the cochlear side by a single layer of squamous cells. In the upper part of the medial wall of the tympanic cavity is the *oval window* (*fenestra ovalis*) which is closed by the foot plate of the stapes. The oval window separates the tympanic cavity from the scala vestibuli of the cochlea.

The **ossicles** (Fig. 26.4) consist of the *malleus* (hammer) with its handle (manubrium) attached to the tympanic membrane, the *incus* (anvil) whose head articulates with the head of the malleus and whose process articulates with the head of the *stapes* (stirrup): the base of the latter fits into the oval window. The ossicles consist of lamellar bone, the stapes containing

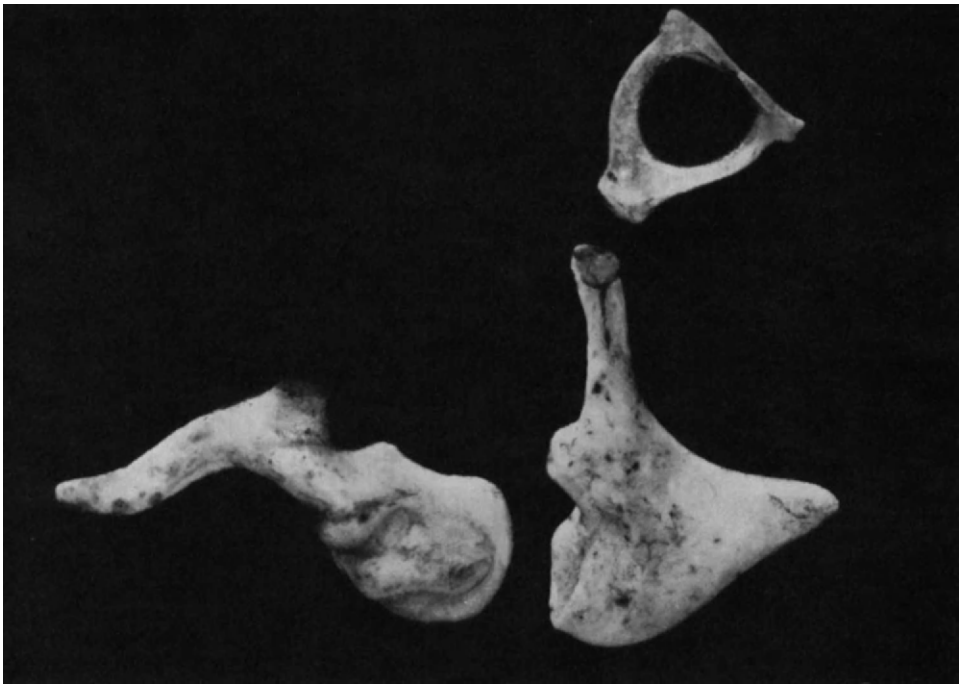

FIG. 26.4. A photograph of the human ear ossicles, from left to right malleus, incus and stapes.

a marrow cavity: the articular surfaces are covered by hyaline cartilage. The covering periosteum fuses with a very thin layer of connective tissue, which is in its turn covered by squamous epithelium continuous with that of the tympanic cavity.

The ear ossicles are responsible for the transmission of sound vibrations from the tympanic membrane to the fluid (endolymph) in the cochlea. There is a very great dissimilarity in the acoustic resistance of air and the cochlear fluids; the ossicles function as a lever system to change a small pressure difference over a large area of the tympanic membrane to a much larger pressure exerted over the small area of the foot plate of the stapes and so overcome the dissimilarity in acoustic resistance between air and endolymph.

The **pharyngo-tympanic (Eustachian) tube** has a narrow lumen surrounded at the tympanic end by bone and supported for the rest of its course by a hook-shaped plate of hyaline cartilage encircling its upper and medial sides. The epithelium is columnar and ciliated in the bony portion, becoming stratified and ciliated, with some goblet cells and tubulo-alveolar glands at

the pharyngeal end. The underlying stroma is infiltrated with lymphocytes and contains both mucous and serous glands. This tube serves to keep the air pressure in the middle ear at atmospheric level, so equalizing the pressure on the two sides of the tympanic membrane.

The **Muscles.** The tensor tympani and stapedius muscles consist of striated fibres. The former maintains the tone of the tympanic membrane, and the latter is concerned with the articulation of the ossicles. (Very loud sounds cause these muscles to contract reflexly, and so alter the amplitude of the vibrations, thereby protecting the delicate cochlea from mechanical damage.)

THE INNER EAR (LABYRINTH)

The inner ear consists of the bony labyrinth containing perilymph in which is suspended the membranous labyrinth containing endolymph. The bony labyrinth, hollowed out of the petrous part of the temporal bone, comprises the *vestibule* (housing the *saccule* and *utricle* of the membranous labyrinth), the *semicircular canals* and the *cochlea*: it is lined by a layer of flattened cells that covers the periosteum. The membranous labyrinth (Fig. 26.5) consists of a

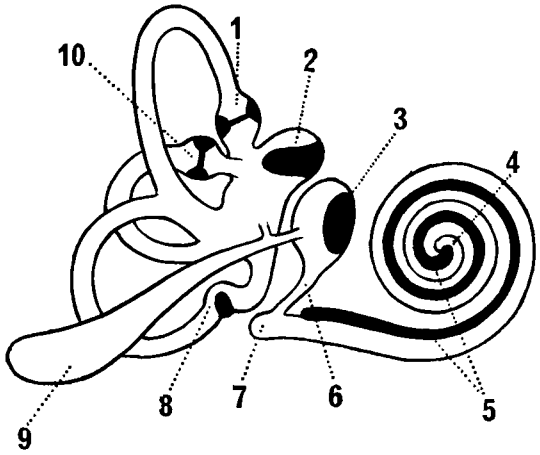

FIG. 26.5. A diagram of the membranous labyrinth with the sensory epithelium in black. 1, ampulla of superior semicircular canal; 2, utricle; 3, saccule; 4, cochlear duct; 5, organ of Corti; 6, ductus reuniens; 7, blind end of cochlear duct; 8, ampulla of posterior semicircular canal; 9, endolymphatic sac and duct; 10, ampulla of the lateral semicircular canal.

fibrous wall lined by epithelium of ectodermal origin, but the wall is modified in those parts in which nerve endings are found, namely, the cristae of the ampullae, the maculae of the utricle and saccule, and the cochlea. In a few places the outer fibrous wall of the membranous labyrinth adheres to the periosteum of the bony labyrinth, but usually the two are separated by spaces (containing perilymph) that are bridged across by strands from the periosteum consisting of connective tissue covered by flat connective tissue cells and carrying blood vessels. (These perilymphatic spaces are homologous to the subarachnoid spaces of the meninges, and the perilymph corresponds with cerebrospinal fluid.)

The **Semicircular Canals.** In cross section the membranous canals are oval and lie excentrically in the bony canals (Fig. 26.6). The thin layer of connective tissue of the wall is continuous externally with the trabeculae traversing the perilymphatic space: internally it is separated by a basal lamina from the lining squamous epithelium. On the inner side of the canal the epithelium is columnar. In each ampulla is a *crista ampullaris* (Fig. 26.7), a prominence projecting from the floor. Here the epithelium is columnar and consists of two kinds of cells: the sustentacular cells, narrow in the middle, wide at each end and with basal nuclei, and the hair cells:

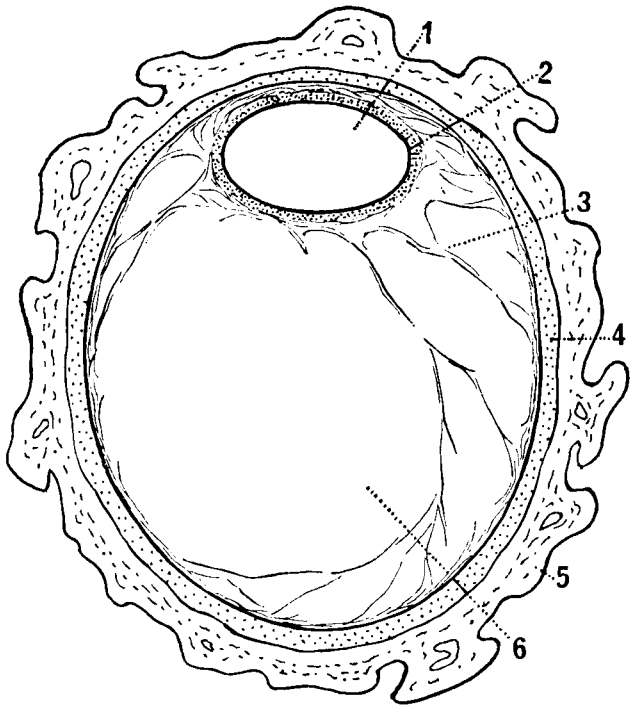

FIG. 26.6. Transverse section of a semicircular canal (diagrammatic). 1, endolymphatic space of the actual membranous canal; 2, epithelium of membranous canal; 3, connective tissue strands; 4, periosteum; 5, bone of bony part of the canal; 6, perilymphatic space.

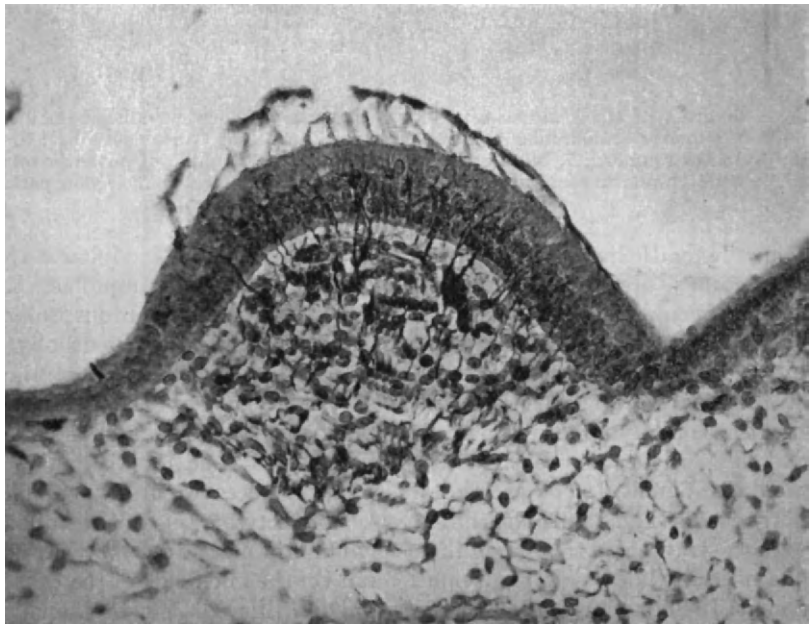

FIG. 26.7. A section through the ampulla of a semicircular canal, showing a crista. Note the nerve fibres ending among the columnar cells. 15 μm.

these latter occupy only the upper half of the epithelium and are flask-shaped with the nucleus in the expansion; they have a tuft of microvilli and one modified cilium on the free surface.

Recent E/M studies have shown that two types of sensory cell can be distinguished; type I has a very extensive nerve ending which envelops the cell like a basket whilst type II cells have a nerve termination which consists of several individual boutons contacting the cell along its length. Covering the surface of the crista is a layer of gelatinous material, the *cupola*, into which the hairs project: the upper layer of jelly contains minute, calcareous otoliths. The nerve fibres ramify among the hair cells. In the area of the crista the connective tissue of the wall is thickened and the intercellular substance is very firm.

The cristae in the ampullae of the semicircular canals provide nervous impulses which reflect any angular acceleration of the head; this type of movement causes movement of the endolymph in the canal leading to a deflection of the cupola. As the processes of the hair cells are embedded in the cupola, any such movement will therefore bend them and stimulate the sensory hair cell.

Utricle and Saccule

The walls are similar in structure to those of the semi-circular canals. In both utricle and saccule is a patch of sensory epithelium, the *macula*: the structure of the macula is essentially the same as that of the crista of the semi-circular canals. The maculae in the utricle and the saccule are concerned with the perception of stimuli arising from linear acceleration of the body.

Cochlea (Fig. 26.8)

The bony cochlea consists of a central axis, the *modiolus*, round which a spiral bony canal winds two and a half times. Projecting from the modiolus is a bony shelf that stretches

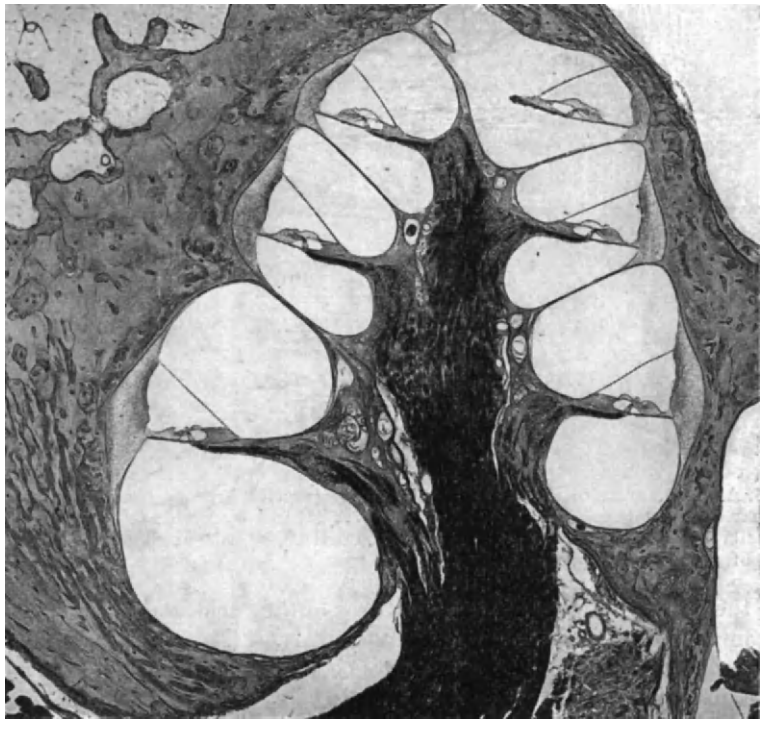

FIG. 26.8. Section through the cochlea of a dog; preparation and photograph by the Ferens Institute of Otology, courtesy of Dr. C. S. Hallpike, F.R.S. 500 μm.

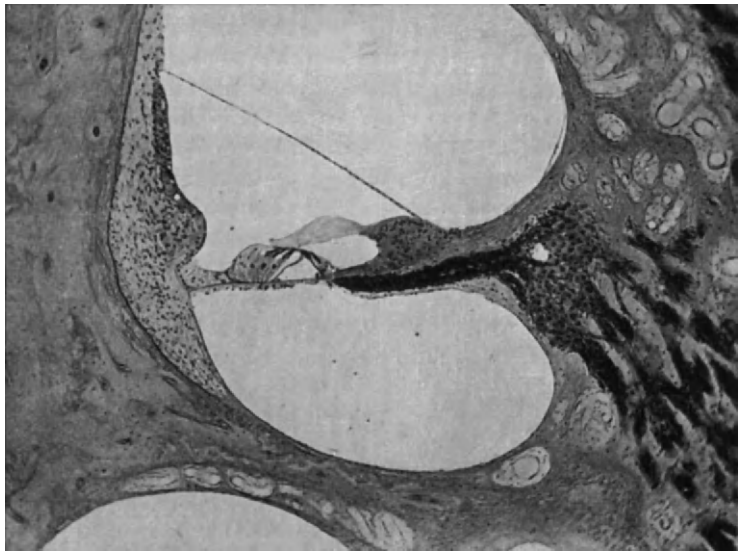

FIG. 26.9A. A section through the cochlea of a cat; preparation and photograph by the Ferens Institute of Otology, courtesy of Dr. C. S. Hallpike, F.R.S. 60 μm.

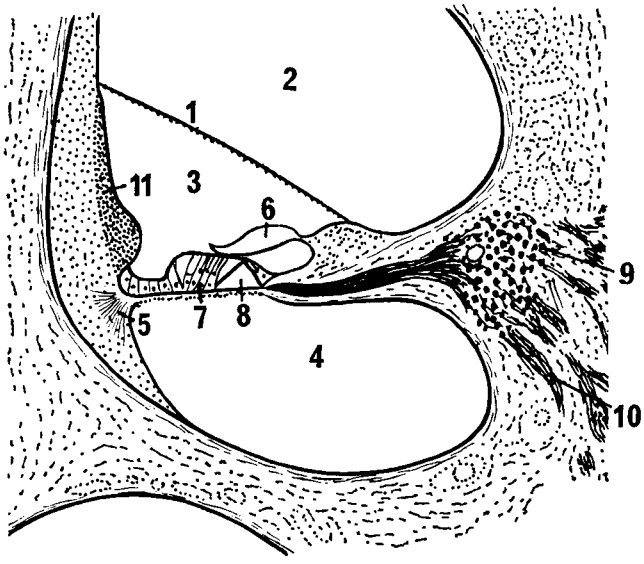

FIG. 26.9B. A key diagram of Fig. 26.9A. 1, Reissner's (vestibular) membrane; 2, scala vestibuli; 3, cochlear duct (scala media); 4, scala tympani; 5, spiral ligament; 6, tectorial membrane; 7, hair cells on basilar membrane; 8, tunnel of Corti; 9, spiral ganglion of cochlear nerve; 10, fibres of cochlear nerve; 11, stria vascularis.

partly across the bony canal and follows the turns of the cochlea as the *spiral osseous lamina*. The shelf is completed across the gap to the outer bony wall by the *membranous spiral lamina* or *basilar membrane*: at the line of attachment of the basilar membrane to the outer wall of the cochlea the periosteum is greatly thickened, forming the *spiral ligament*. In this way the bony canal is divided into two parts, an upper *scala vestibuli* and a lower *scala tympani* (see Figs. 26.8, 26.9): these contain perilymph and communicate with one another at the distal end

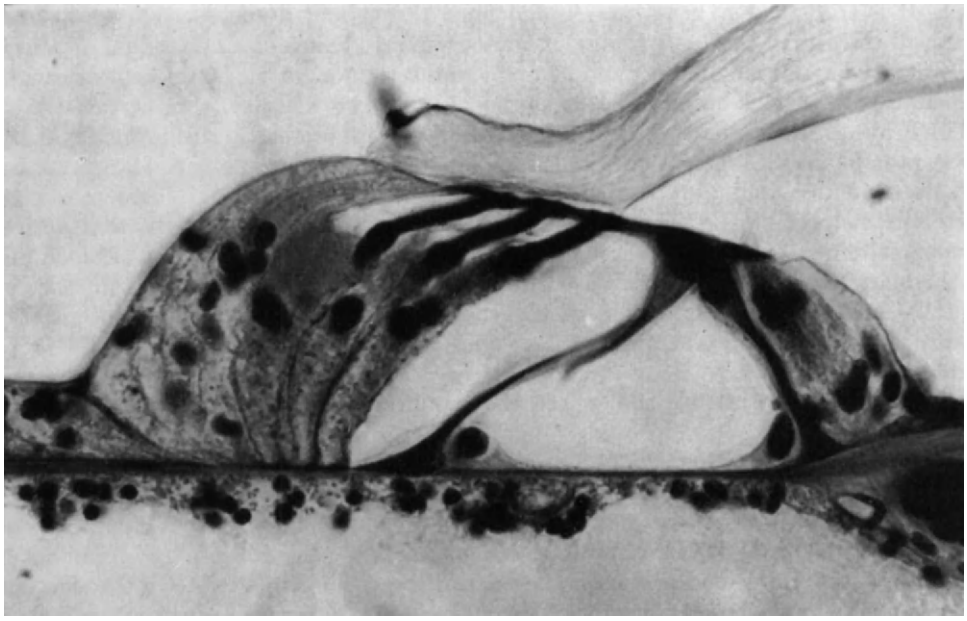

FIG. 26.10A. Photomicrograph of a section through the organ of Corti and the tectorial membrane. 15 μm.

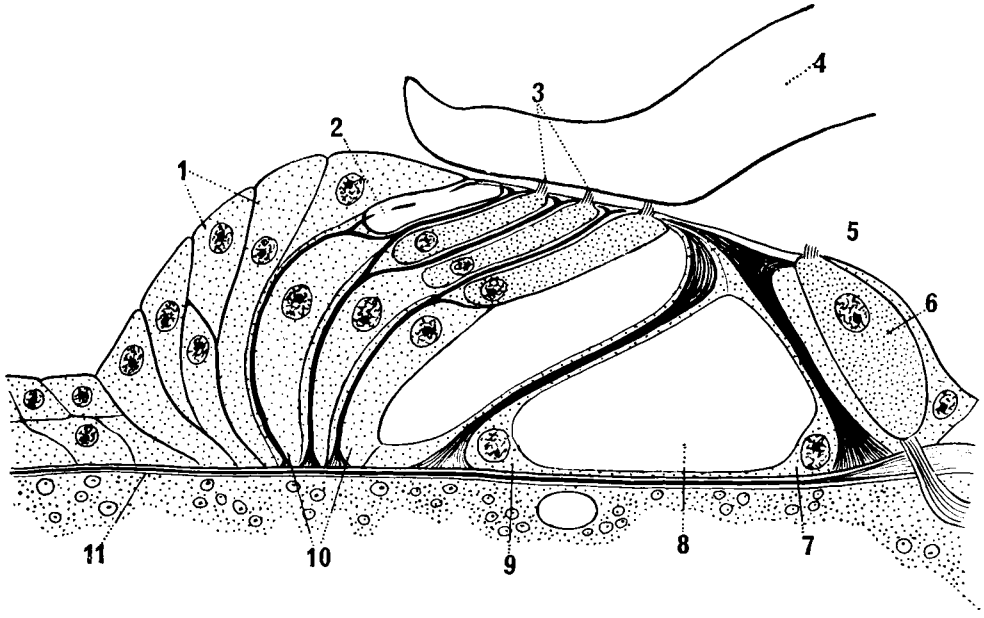

FIG. 26.10B. A key diagram of Fig. 26.10A. 1, cells of Hensen; 2, outer tunnel; 3, outer hair cells; 4, tectorial membrane; 5, internal spiral tunnel; 6, inner hair cell; 7, internal pillar cell; 8, inner tunnel; 9, outer pillar cell; 10, outer phalangeal cells; 11, basilar membrane.

of the cochlea by the *helicotrema*. There is a communication between the subarachnoid space and the scala tympani by a minute canal (the aqueduct of the cochlea).

Near the middle of the bony cochlear tube, lying between the scala vestibuli and the scala tympani, is the membranous *cochlear canal* (*scala media*, *ductus cochlearis*) containing endo-

lymph. It is triangular in cross-section and lined throughout by ectodermal epithelium: its lower wall consists of the bony and membranous spiral laminae, its upper wall of *Reissner's membrane* (vestibular membrane), and its outer wall is the wall of the bony cochlear canal. The epithelium covering the outer wall is thickened and is very vascular (*stria vascularis*).

The E/M has shown that several different cell types are present in this layer; the marginal cells seem to be the most active ones and are characterized by the presence of large numbers of basal mitochondria and very extensive plasma membrane infoldings. These cells possibly secrete the endolymph, but the presence of the cellular infoldings suggest that active ion transport is taking place. The endolymph may well be initially a transudate of blood plasma and the marginal cells of the stria vascularis may be responsible for the ion transport which maintains the unusual composition of the endolymph (it possesses a high concentration of K^+ ions and a low concentration of Na^+ ions, the opposite of that normally found in plasma).

The endolymph ultimately may reach the subdural space by way of the endolymphatic duct and sac.

Reissner's membrane (Fig. 26.9), separating the cochlear canal from the scala vestibuli, consists of connective tissue covered on both sides by a single layer of cells. The *basilar membrane* (Fig. 26.10) consists of flexible fibres embedded in a homogeneous ground substance; it stretches tightly from the bony spiral lamina outwards to penetrate into the spiral ligament: the length of the fibres increases from the base of the cochlea upwards. On the side next to the scala tympani the basilar membrane is covered by connective tissue and a single layer of flat cells. On the cochlear side of the membrane the covering is epithelial and highly differentiated: the cells of the outer part are columnar, but those of the inner part are modified into the organ of Corti.

Organ of Corti (Figs. 26.9, 26.10)

The spiral organ of Corti extends throughout the two and a half turns of the cochlea, and consists essentially of supporting cells and hair cells, the latter being neuro-epithelial in nature. The pillars of Corti are arranged as two rows of cells—inner and outer pillars: their bases contain the nuclei and lie some distance apart on the basilar membrane, while their heads "articulate" together, so that the diverging pillars enclose a spiral canal, the *tunnel of Corti*. The tunnel contains a gelatinous material and is bridged by fine nerve fibres. On either side of the pillars of Corti lie the *hair cells*, a single row (inner hair cells) next to the inner pillar and four rows (outer hair cells) outside the outer pillar (Fig. 26.10). The hair cells are short and do not reach the basilar membrane but are supported by being enclosed in the so-called *phalangeal cells* (Fig. 26.11). The hair cells each possess a number of microvilli ("hairs") arranged on the superficial surface of the cell in a W or U shape. The tips of the hairs are embedded in the tectorial membrane. The phalangeal cells (the supporting cells of Deiters) not only enclose the cell bodies of the hair cells but also send long cytoplasmic processes in between them, terminating in an apical umbrella-like expansion (Fig. 26.11). This expansion is inserted between the free edge of the hair cell which it is enclosing and the hair cell in the next row. As well as ensheathing the hair cells, the E/M has shown that the phalangeal cells also enclose both afferent and efferent nerve fibres which are passing to the hair cells.

Outside the outer row of hair cells are seven or eight rows of columnar supporting cells, the *cells of Hensen* (Fig. 26.10).

The *tectorial membrane* is a non-nucleated jelly-like substance arranged as a membrane which is attached to the osseous spiral lamina (Fig. 26.10). It extends outwards to rest lightly on the organ of Corti at the outer limit of the hair cells.

The organ of Corti is responsible for the initiation of nerve impulses which are conveyed to the C.N.S. by the auditory nerve and which are interpreted as sound. For details of the manner in which pitch and loudness are appreciated a text book of physiology should be consulted.

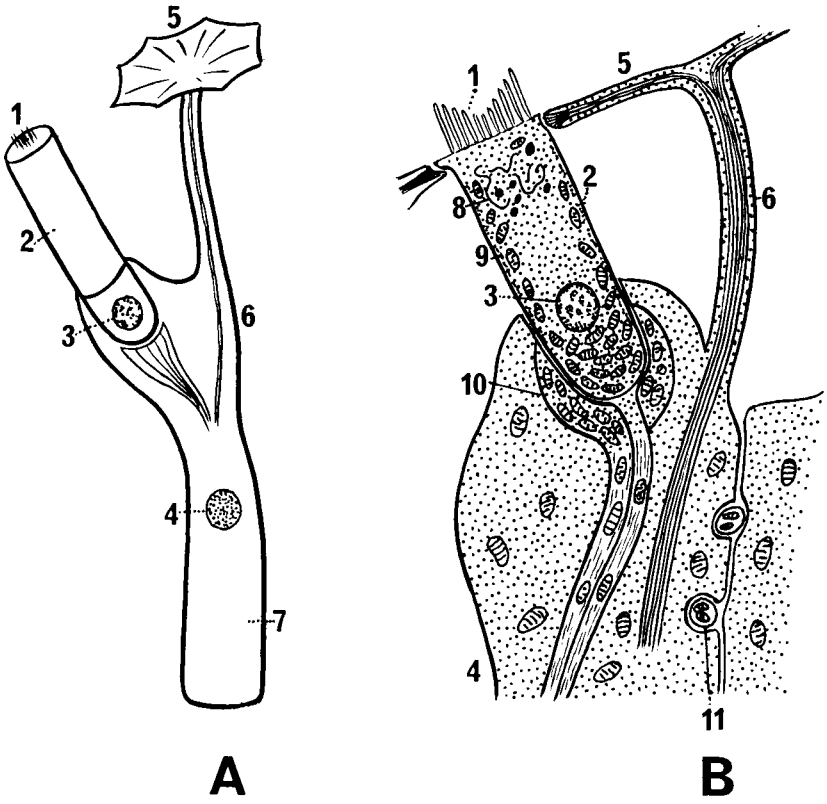

FIG. 26.11A. Diagram of an outer hair cell and the associated phalangeal cell (supporting cell of Deiters). 1, hairs; 2, outer hair cell body; 3, nucleus of hair cell; 4, nucleus of phalangeal cell; 5, phalangeal process or "umbrella"; 6, fibrils in the stalk of the phalangeal cell process; 7, phalangeal cell body.

B. A diagram of the appearance of an outer hair cell and the phalangeal cell when examined with the E/M. 1, hairs; 2, cell body of hair cell; 3, nucleus of hair cell; 4, cell body of phalangeal cell; 5, "umbrella" of phalangeal cell; 6, process of phalangeal cell containing microtubules; 8, granular ER in hair cell; 9, mitochondria in hair cell; 10, efferent nerve fibre ending; 11, branches of the cochlear nerve in section.

Blood Vessels

One branch of the auditory artery supplies the bony labyrinth and another the membranous labyrinth; the latter branches again dividing into vestibular and cochlear divisions. The distribution is in general with the nerve fibres: in particular the cristae and maculae are richly supplied, also the inner part of the basilar membrane and the inner wall of the cochlear canal. The veins take a different course from the arteries; in the cochlea the arteries are mostly in the walls of the scala vestibuli and the veins in the walls of the scala tympani.

Lymphatics

There are no true lymphatics, but the fluid is drained into the perilymphatic spaces which are connected with the subarachnoid spaces of the brain. There is also some drainage by the spaces in the adventitial sheaths of the blood vessels and nerves.

Nerves

The vestibular branch of the eighth nerve supplies the vestibular part of the inner ear, sensory nerve endings being found in the maculae and the cristae. The cochlear branch

supplies the cochlea. Typical bipolar nerve cells form the spiral ganglion of the cochlea in the bony spiral lamina; the axons of these cells leave by the modiolus: the myelinated dendrites pass outwards, lose their myelin sheath and form bundles running parallel to the tunnel of Corti and giving off branches that end in fine (sensory) terminals on the hair cells.

References for further detailed study:

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APPENDIX 1

CERTAIN ABNORMAL VARIATIONS IN MINUTE STRUCTURE

In the histological examination of tissues and organs it is not unusual to find some variation that falls outside physiological limits, and this appendix is added to help the student to recognize such changes: it is not intended in any way as an introduction to the systematic study of morbid histology.

(1) Post-mortem Changes

After death the tissues break down very rapidly, although the process can be delayed by cooling. A raised body temperature at the time of death hastens this deterioration. Further, if oedema was present, the cells and tissues after death imbibe water and swell, and if bacteria were present these tend to multiply quickly. In all cells the mitochondria are the first structures to break down. The alimentary canal undergoes particularly rapid breakdown. The usual post-mortem changes met with are the following.

(*a*) **Nucleus.** The nuclei shrink and stain uniformly and deeply: at first the staining affinity is normal, i.e. basiphil, and later it becomes acidophil. Later still the nuclei fail to stain.

(*b*) **Smooth Muscle.** The cells swell in the middle and stain deeply and uniformly with eosin.

(*c*) **Epithelia.** Epithelial cells desquamate, and are found lying freely in the lumen of the tube that they line. This occurs rapidly in the alimentary tract, the gall-bladder and in the prostate and seminal vesicle. The endothelial lining of the blood vessels also desquamates very quickly after death.

(*d*) **Red Blood Corpuscles.** The haemoglobin tends to dissolve out quickly. Formalin produces a precipitate of black granules with haemoglobin.

(2) Necrosis

Necrosis is local death of cells while the surrounding cells remain alive.

Local death of the cells may occur quite normally in the body, the worn-out cells being then replaced. But areas of necrosis may result from many pathological processes. Necrotic tissue shows the following peculiarities.

(*a*) **Cells.** They appear fused as the boundaries disappear. The protein contents coagulate and lipid granules often appear. Sometimes the cells swell, liquefy and burst (cytolysis).

(*b*) **Nucleus.** The chromatin contracts into an irregular, darkly staining lump (pyknosis): it may fragment (karyorrhexis), or dissolve and disappear (karyolysis).

(3) Inflammation

Inflammation is "the sequence of changes which occurs in a living tissue when it is injured provided the injury is not of a degree sufficient to kill the tissue outright" (Burdon Sanderson). The changes are classed as acute or chronic according to the stimulant.

The four cardinal signs of acute inflammation are redness, rise of temperature, swelling and pain. These symptoms are produced as follows. The redness is due to acute hyperaemia, as the injurious agent causes local vasodilation of the arteries supplying the part. The temperature rises because of the increased blood flow. After a short time the blood stream slows down in

the dilated vessels, even to below the normal rate: thus the cells from the axial stream come to lie against the vessel walls. This is followed by an increased leakage of plasma, the oedematous fluid serving to dilute any toxic substances. The exudation is the cause of the swelling, and also of the pain as the exudate presses on the nerves. Very shortly the blood cells begin to migrate through the vessel walls (diapedesis), the leucocytes moving actively while the red corpuscles are pushed out. There is a general leucocytosis, due to humoral stimulation of the marrow, and the damaged area becomes filled with polymorph leucocytes. An accumulation of leucocytes fighting bacteria produces an abscess, the contained pus consisting of bacteria, degenerated leucocytes and tissue debris.

The neutrophil polymorphonuclear leucocytes play the most important part in acute inflammation, reaching the damaged area in large numbers but not multiplying there. They cannot by themselves however finish the process of reaction and repair. Large mononuclear cells and mobilized macrophages act as scavengers, carrying the débris into the lymphatics so that the fluid can be filtered of foreign matter in its passage through the lymph nodes. Toxins may be drained away in the same way, and it is common to find the lymph nodes enlarged to deal with these substances. When the inflammatory process is chronic or of long duration, lymphocytes are usually the predominant type of leucocyte, particularly at the periphery of the lesion: plasma cells also appear.

The fluid exudate contains fibrinogen, and fibrin may form in varying amounts due to the enzymes set free by the disintegrating leucocytes: this occurs most often on surfaces.

(4) Repair

Repair includes the removal of foreign bodies, the organization of fibrin and the healing of destroyed tissues by regeneration of cells. Foreign bodies are removed by special activities on the part of leucocytes and macrophages, including the formation of foreign body giant cells round insoluble substances. Repair processes appear to be stimulated by vitamin C.

By *organization of fibrin* is meant the replacement of the fibrin of an exudate by connective tissue. The local fibroblasts proliferate, elongate and give off fibrils, so giving rise to an irregular network that also contains inflammatory cells. At the same time capillary blood vessels grow out from pre-existing vessels into the fibrin and the line of demarcation between tissue and coagulum disappears. Other capillary loops grow out from the first, advancing right into the fibrin mass. This is the *granulation tissue* and is temporary in character. The fibroblast cells then become spindle-shaped, orientated at right angles to the budding capillary loops; they thus form bands of fibrous tissue which ultimately obliterate the capillaries. In this way the whole mass is replaced by dense fibrous tissue that contracts to produce the scar. (In wounds of the skin if the tissue continues to grow after healing is completed, an irregular mass of scar tissue called *cheloid* is produced.)

(5) Catarrhal Inflammation

This type of inflammation involves the desquamation of the cells of lining membranes and stimulation of any glands in the membrane, so increasing their secretion. The general reaction is similar to that in other forms of inflammation.

PROTECTIVE MECHANISMS IN THE BODY

The body is continually exposed to conditions that might lead to the damage of its cells and tissues, but it is well adapted to prevent the entry of injurious substances into the actual tissues, and also to deal with such substances if they should enter from without or be produced within. The following account deals chiefly with the microscopical aspect of the subject and is meant to be suggestive rather than exhaustive.

A. Usual Sources of Entry of Injurious Substances

(1) **Solid particles**—such as bacteria, dust, carbon, etc.

(*a*) Nose—passing to the respiratory organs.
(*b*) Mouth—passing to the respiratory organs and the alimentary tract.
(*c*) Anus—passing to the alimentary tract.
(*d*) External genital apertures—passing to the genital tubes and organs.
(*e*) External urinary aperture—passing to the bladder, ureters and kidney.
(*f*) Pharyngo-tympanic tube (or through damaged tympanic membrane)—passing to the middle ear, mastoid air cells and possibly the brain.
(*g*) Skin, by the hair follicles, sebaceous and sweat gland ducts and the nipple, or through damaged skin—passing to the underlying tissues and into the blood and the lymph vessels.
(*h*) Damaged gut wall—passing to the peritoneal cavity.

(2) **Chemical poisons** (in solution).

(*a*) Absorbed by the gut, and reaching the blood stream.
(*b*) Absorbed by the skin, and reaching the blood stream, e.g. D.D.T.
(*c*) By the sheaths of certain cranial nerves, particularly olfactory, optic and auditory, and reaching the subarachnoid space and the brain. (E.g. diphtheria toxin, poliomyelitis virus.)
(*d*) Made by bacteria already present, and passing into the tissues, the blood and the lymph streams.

B. Prevention of Entry

The most usual and easy paths for the entry of these substances are provided with a special structure, both gross and minute, in order to make such entry difficult. A few examples of such modifications are given below.

(1) **Skin.** The skin is provided with a stratified epithelium that is greatly thickened where friction is great: in this way the underlying living cells of the epidermis are protected, and are easily repaired if damaged. The openings of the sweat ducts are protected by the continuous outward flow of fluid. In the hair follicles the sebaceous gland openings are not so well protected, and these provide the most usual place for infection to occur.

(2) **Respiratory Tract.** On entering the nose the air is roughly filtered by the vibrissae. The epithelia are covered by a layer of mucus and any small bodies are entangled in the mucous secretion. The whole of the tract down to the respiratory bronchioles is provided with mucin-secreting cells and cilated cells, so that there is a constant outward current to sweep out inhaled particles. If foreign bodies should reach the alveolar tissue, the phagocytic dust cells engulf them and deposit them in the lymphoid tissue closely associated with the larger blood vessels and the bronchioles.

(3) **Alimentary Tract.** The mouth, the oesophagus and the anal canal, all places where friction by solid contents is liable to occur, are lined by stratified epithelium: in addition, the oesophagus and particularly the anal canal are well provided with mucous glands whose mucin also helps to minimize any friction. The distribution of the mucin-producing cells throughout the whole tract is associated with the need for surface protection, both physical and chemical. There is much diffuse lymphoid tissue in the mucous and submucous coats throughout: definite lymphoid nodules are found in increasing numbers from the mouth to the anus, and also in those places where a temporary stasis of the contents occurs, such as the oropharyngeal junction and the lower end of the ileum: this distribution is clearly associated with the prevention of entry into the body of all types of poisonous material.

(4) **Blood—Brain Barrier.** The cerebrospinal fluid, which is a transudate of the blood

plasma, has to pass through the cells of the choroid plexus before it can enter the ventricles of the brain. The cells of the choroid plexus, therefore, have a chance to modify the transudate so that any toxic substance present in the blood is excluded from the C.S.F. and so from coming into close contiguity with the nervous tissue. All the small capillaries in the C.N.S. have an investment of glial cells (see p. 167) so that again a modifying influence may be exerted on substances which leave the blood stream and pass into the nervous tissue.

C. Methods Whereby the Body Deals with Infection or Damage to Tissues

(1) **Macrophages** (Histiocytes). Connective tissue responds as a whole, but probably the most important of the reacting cells are the macrophages. These cells have been described on p. 71. All the more actively phagocytic cells of the body except the polymorph leucocytes are included. As these cells are components of all connective tissue their distribution in the body is universal. They exert a local protective function, by collecting débris and removing it. The "fixed" macrophages are rapidly mobilized in response to a stimulus, forming some of the polyblasts in tissues and exudates. After engulfing the foreign matter they may degenerate, or they may be eliminated through the intestinal wall or the wall of the respiratory tract, being ultimately lost in the mucous secretions. Such a reaction may be physiological, and of normal occurrence: a pathological inflammation is a more intense local reaction to noxious stimuli (see also p. 427).

(2) **Polymorphonuclear Leucocytes** (microphages). These cells are transported in the blood stream: as almost all tissues are supplied by blood, they constitute an important line of defence. Most varieties of the leucocytes are amoeboid, and can pass through blood vessel walls. They are drawn to the site of injury or infection by some process which may possibly be of the nature of chemical attraction.

(3) **Blood Filters.** In certain organs the blood is in such close and intimate relation to the reticulo-endothelial tissues that a "filtration" of solid particles occurs. The process is probably one of adsorption, and the particles are dealt with by the histiocytes in contact with the blood. The organs chiefly concerned are the spleen, lymph nodes, the liver and the red marrow.

(4) **Lymph Filters.** All lymph nodes on the course of lymphatic vessels act as lymph filters. The lymph percolates through the node, and the macrophages, reticular cells and cells lining the lymph channels remove any solid foreign matter. At the same time the lymphocytes deal with any toxic substances in solution in the lymph.

(5) **Antibody Formation.** Foreign substances of a protein nature stimulate the production by the body of antibodies which are γ globulins. For details of the production of antibodies and how they react in the defence of the body a text book of immunology (such as "Immunology for Students of Medicine" by J. H. Humphrey and R. G. White. Blackwell, 3rd edn., 1968) should be consulted.

APPENDIX 2

HISTOLOGICAL METHODS*

There are two approaches available for the investigation of the minute structure of tissues, namely the direct observation of living cells and the making of permanent preparations of dead cells.

Examination of fresh tissues has the great advantage of yielding information as to structure in conditions most closely resembling those of the body; it may be also a method of great value where speed of preparation is essential for diagnosis. On the other hand, the preparations are not permanent, and they rapidly deteriorate unless subsequently fixed. Permanent preparations have the advantage of providing a record of structure that should last unchanged for years: in addition, the fixation necessary for such preparations makes it possible to apply a great variety of histological processes to the tissue, thus allowing of a very complete investigation. A combination of the two methods is made use of in vital and supra-vital staining (see below). As no fixative preserves all the structures of the cell, and as staining reactions are also selective, it is essential to know the methods of fixing and staining before describing the appearance of any preparation: any observation should always be checked by various techniques. In recent years certain new technical methods have come into use.

(*a*) The freezing-drying method of preparation, in which the tissue is rapidly frozen and then dehydrated in a vacuum, thus giving less distortion of the tissue than other fixation methods.

(*b*) Biopsy methods, in which small portions of tissue are removed surgically from the living subject for histological examination.

(*c*) The use of new types of microscope.

(*d*) The ultra-centrifuge, whereby homogenates containing components of the cell can be separated into layers according to their specific gravity, and thus identified and examined.

(1) FRESH PREPARATIONS

(*a*) Isolated Cells in a Fluid Medium

Examine drop on slide by dark ground or phase contrast microscopy.
If too rich in cells, dilute with isotonic solution.
If too poor in cells, centrifuge and examine sediment.
Do not dilute if crystals are present.

(*b*) Organs

Before examination the individual cells must be separated from one another or sections prepared.

(1) Isolation of cells

Film preparation e.g. for blood.
Imprint preparation e.g. for bone marrow or lymphoid tissues.
Separation by means of a mechanical blender.
Enzymic digestion with trypsin or Pronase solutions.
Maceration in 30 per cent alcohol or 1/5000 chromic acid, etc.

* A selection only is given here of methods likely to be of most value to students. For further information consult "A Handbook of Histopathological Technique" by C. F. A. Culling, "Staining Animal Tissues" by E. Gurr, or Peacock's "Elementary Microtechnique" (ed. Bradbury).
The formulae of solutions mentioned are given at the end of the appendix.

(2) Preparation of sections
Cut freehand.
Cut by cryostat.
Cut on conventional freezing microtome, sometimes after soaking in gum for additional support.

(*c*) Tissue Culture

Cells can be grown as an aseptic culture in a suitable medium consisting of a nutritive phase containing embryo extract, and also a solid phase for support: the cells resulting and their differentiations can then be examined.

(*d*) Use of Warm Stage for Examining Living Objects

The movements of living cells can be examined in a hanging drop preparation on a warm stage.

(*e*) Vital Staining

Some dyestuffs, e.g. lithium carmine, trypan blue, diluted indian ink, can be introduced intravenously into the living animal; certain cells pick up the stain electively. (For example, the distribution of reticulo-endothelial cells can be studied by this method.)

(*f*) Supravital Staining

Some dyestuffs can be used to stain certain living tissues for which they have an affinity. (Under some conditions methylene blue will stain nerve endings, and Janus green B and certain tetrazolium salts, mitochondria.)

(*g*) Micro-dissection

Very fine glass needles can be manipulated under the high power of the microscope, so that individual cells can be dissected.

(*h*) Cinematography

Cinematography through the microscope provides a permanent record of cell activity, and also furnishes an aid in the analysis of very slow or very fast movement.

(2) PERMANENT PREPARATIONS

In order to provide permanent preparations the fresh tissue must be fixed.

(*a*) Fixation

Obtain material as soon after death, or remove by biopsy methods if possible to prevent post-mortem change.

Take small pieces, preferably not more than 2 mm. thick.

Requirements in a Fixative

(i) Rapid penetration.
(ii) Production of immediate death of cells.
(iii) Stabilization of structure against further treatments.

Artifacts Due to Defective Fixation

Shrunken protoplasm, producing spaces.
Appearances of granules and fibres not previously present.

Fixative Methods

(i) Formalin mixtures.
  (*a*) 10 per cent in isotonic NaCl solution (or in phosphate buffer)—suitable for all tissues: preserves lipid.
  (*b*) In combination with Müller's fluid (below).
  (*c*) Heidenhain's "Susa" mixture.

(ii) Alcohol mixtures.
  (*a*) 96 per cent or absolute alcohol—suitable for films or preserving granules and bacteria. Causes shrinkage due to dehydration.

(iii) Chromic acid mixtures.
  (*a*) Müller's fluid. Suitable for large pieces of brain or cord. Acts as mordant, but makes tissues brittle.

(iv) Mercuric chloride mixtures.
  (*a*) Zenker's fluid. Suitable for microanatomy, but mercury salt precipitate must be removed by treatment with iodine in potassium iodide before staining.

(v) Osmium tetroxide mixtures.
  (*a*) Osmium tetroxide, 1 per cent. Excellent for granules and nuclei. Penetrates very slowly. Much used for electron microscopy.
  (*b*) Marchi's fluid. For degenerating nerve fibres (see below).

(vi) Picric acid mixtures.
  (*a*) Bouin's fluid. Suitable for most tissues, which it fixes rapidly. Remove by washing in alcohol, not water.
  (*b*) Picrocarmine. A combined fixing and staining mixture. Useful for teased preparations and for nervous tissues stained in bulk.

(*b*) Decalcification

Bones, teeth and other structures containing calcareous deposits must be decalcified before it is possible to cut sections from them. Decalcification should be done *after* fixing. The following points should be observed:

(1) Small pieces of tissue to be taken.
(2) Large excess of decalcifying fluid to be used.
(3) Fluid to be changed frequently during decalcification.
(4) Tissue to be left only until decalcification is completed. (Test by X-rays, or by pricking with a fine needle.)
(5) Tissue to be washed very thoroughly in alkaline running (tap) water after decalcifying.
(6) Staining time to be prolonged.

The best fixative for general use is 10 per cent formalin.
The decalcifying fluids in common use are:

(1) Dilute nitric acid.
(2) Trichloracetic acid mixture.
(3) Ebner's fluid.
(4) Ethylene diamine tetra acetic acid (EDTA).

(*c*) Section Cutting

In order to cut sections the tissue must be hardened e.g. by freezing, or embedded in some supporting medium. The supporting medium may be:

(i) Watery—e.g. gum (which is subsequently frozen).
  gelatin (which sets hard when cold).

(ii) Anhydrous—e.g. celloidin.
paraffin wax.

The details of each process are enumerated below.

Sections are cut on a microtome, a machine whereby the thickness of the sections is regulated automatically. There are three types of microtome in common use.

(i) Cambridge rocker type, with knife fixed and block moving vertically.
(ii) Sledge type, usually with block fixed and knife moving horizontally.
(iii) Rotary type, with knife fixed and block moving vertically.

A cryostat, i.e. a freezing cabinet with a rocker microtome inside it at about −30°C, is often used for the preparation of sections for histochemical procedures.

The following are the details of the three routine methods:

(i) Gum Freezing Method

Fix tissue, about twenty-four hours.
Rinse in water.
Put into gum solution (gum acacia, 2g; sucrose, 60g; distilled water, 200ml.), at least thirty minutes.
Put on block of carrier of freezing microtome (sledge type) and freeze with bursts of carbon dioxide gas; keep plenty of gum solution on the block.
Transfer sections as cut to 25 per cent alcohol to remove the gum.

(ii) Paraffin Wax Method

Fix tissue, about twenty-four hours.
Wash in running water, twenty-four hours.
Transfer to 70 per cent alcohol, at least one hour. (Tissue can be left safely at this stage over-night.)
Transfer to 96 per cent alcohol, about two hours.
Dehydrate completely in absolute alcohol (two treatments of about one hour each).
Clear in xylol, giving one change, and leaving until transparent.* (Chloroform, benzene, oil of cloves, oil of cedarwood may be used as alternatives to xylol.)
Tissue must not be left for more than about two hours in any of these fluids except cedarwood oil, in which they can remain for a few days without harm.
(If the tissue does not "clear," it is not completely dehydrated: in this case return to absolute alcohol.)
When clear, transfer to melted paraffin wax of melting point 52°C. The wax is kept at this temperature in a thermostatically controlled bath. Give two changes of wax, with a total stay of about three hours in the bath.
Make a "cell" of suitable size from two L-shaped metal blocks on a surface of glass: fill it with fresh, melted wax, quickly embed the block of tissue, orientating it carefully. Cool rapidly by immersing in a bowl of very cold water. Leave block in cold water for at least two hours.
Wax blocks should be stored in a cool place.
Trim wax block to a rectangular shape.
Cut sections. These should come off the knife in a "ribbon": collect on shiny paper: cut sections apart with a sharp scalpel. Mount sections by placing them on a pool of dilute albumen solution on a microscope slide. Heat on a hotplate at about 45°C to expand the sections. Drain off the surplus albumen, wipe round the sections and leave overnight in an oven at 37°C to dry completely. Mounting every section in order ("serial sections") is easy by this method, as the ribbon is cut into lengths of as many sections as are to be mounted on one slide, and mounted in order.

* Tissues or sections appear transparent only in fluids that have the same refractive index as themselves.

	Slide 1.		Slide 2.		
Section	1	5	9	13	
	2	6	10	14	
	3	7	11	15	and so on.
	4	8	12	16	

Leave room for marking the slide.

(iii) **Celloidin Method**

Fix, wash and dehydrate as in the paraffin wax method. If large pieces of tissue are being used, leave in absolute alcohol at least twenty-four hours.

Transfer to equal parts of absolute alcohol and ether, twenty-four hours.

Transfer to thin celloidin solution (2 per cent) (well covered) at *least* three days. (Celloidin dissolved in absolute alcohol and ether.)

Transfer to thicker celloidin solution (4 per cent) for the same length of time.

Transfer to thick celloidin solution (8 per cent) for the same length of time.

Transfer to fresh thick celloidin solution in a suitable flat-bottomed dish with a sheet of silver paper at the bottom to prevent sticking. Harden slowly in air by leaving the cover slightly off to allow evaporation of the solvent.

When hard enough just to leave the impress of a finger mark, cut out in blocks, and transfer to 70 per cent alcohol for keeping. If allowed to dry up the block will be spoilt.

Cut sections on a sledge-type microtome, keeping the block and the razor flooded with 70 per cent alcohol.

Transfer sections to 70 per cent alcohol as cut.

(iv) **Electron Microscopy**

For details of the preparation of tissues for electron microscopy, consult works such as those by Kay (Ed.) "Techniques for Electron Microscopy" (1965) or Pease "Histological Technique for Electron Microscopy" (1965).

(*d*) Subsequent Treatment of Sections

(i) **Frozen Sections**

Remove sections from 25 per cent alcohol and stain as desired. This may be done in a watch glass or small dish, transferring the sections from one solution to another as necessary by means of a drawn-out bent glass rod or a fine paint brush.

After staining and washing, mount in an aqueous medium such as glycerin, glycerin jelly, Farrants solution or a commercial aqueous mountant.

(ii) **Paraffin Wax Sections**

Remove all the wax by soaking the slides bearing the sections in xylol for several minutes.

Remove the xylol with absolute alcohol.

Take down through descending strengths of alcohol to the strength suitable for the stain or to water if the stain is in aqueous solution. After staining and washing, take up through ascending strengths of alcohol.

Dehydrate completely in absolute alcohol.

Clear in xylol: (if the section does not clear it is not completely dehydrated).

Mount in a synthetic resin such as DPX.

(iii) **Celloidin Sections**

Take sections from 70 per cent alcohol as required and stain by any method that does not involve the use of absolute alcohol (which dissolves the celloidin).

After washing, take up through ascending strengths of alcohol to 70 per cent alcohol.
Clear in phenol xylol.
Mount in synthetic resin.

(*N.B.* The celloidin support is present in the final mounted specimen.)

Summary of Advantages and Disadvantages of these Three Methods of Treatment

Method	Advantages	Disadvantages
Freezing	(1) Very rapid. (2) Lipid is preserved. (3) Easy to carry out.	(1) Sections not very thin. (2) Sections tend to fall to pieces. (3) Serial sections very difficult.
Paraffin wax	(1) Fairly rapid. (2) Excellent for fine detail. (3) Serial sections easy. (4) Sections can be very thin. (5) Blocks easily kept.	(1) Temperature-regulated oven necessary for embedding. (2) Danger of damage by overheating. (3) Lipid is not preserved.
Celloidin	(1) Process carried out in cold. (2) Very large pieces can be used. (3) Excellent for brittle material. (4) Sections do not fall to pieces. (5) Process of embedding requires little attention.	(1) Blocks and sections must be kept under spirit. (2) Serial sections tedious. (3) Sections usually not very thin. (4) Section cutting must be done under spirit.

STAINS AND STAINING*

The value of stains lies in their power of rendering visible structures that were not previously apparent, of differentiating between such structures, and of yielding information as to the chemical nature of certain structures (as in some histo-chemical reactions).

The actual mechanism of staining is complex, and many theories have been brought forward to account for the phenomena involved. Reference should be made to the book listed in the footnote.

Some of the stains in common use for histological purposes may be classified as follows:

(i) Natural Dyes.
Haematoxylin.
Carmine.
Orcein.

(ii) Synthetic Dyes.

A. Acid.
Acid fuchsin.
Eosin.
Biebrich scarlet.
Orange G.

B. Basic.
Basic fuchsin.
Methylene blue.
Toluidine blue.
Aniline blue.
Gentian violet.
Safranin.

* For an account of the rational use of dyes in microscopical work, see J. R. Baker "Principles of Biological Microtechnique" (1958).

Method of Staining

Stains can be used in two ways:

(1) As direct stains.

In this case the stain and the tissue combine directly, e.g. by an ionic linkage, so that by the end of the staining process the tissue has acquired the colour of the dye.

(2) As indirect stains.

In this case a third substance, known as a *mordant*, is required to bring about the union between the tissue and the dye. The mordant combines with the tissue and the dye then interacts with the mordant to form a "fast" colour combination known as a "dye-lake".

Many fixative solutions contain a mordant, e.g. chromic acid. Many staining solutions contain a mordant, e.g. Scott's haematoxylin.

A dye sometimes stains "electively" a single tissue constituent, e.g. resorcin-fuchsin for elastic fibres: this is a *differential* stain. Most dyes, however, stain all the constituents to varying degrees: this is a *diffuse* stain. In the latter case there are two ways in which staining can be carried out to obtain the greatest amount of information.

(1) *Progressive Staining.* The dye is allowed to act until the affinities of certain tissue constituents are satisfied and then the process is stopped.

(2) *Regressive Staining.* The tissues are over-stained and the dye is then washed out from all constituents except those for which it has the strongest affinity. Such differentiation can be carried out by means of acid alcohol, an aniline mixture, or frequently by 75 per cent alcohol: the choice depends on the stain.

In order to obtain the best results from staining, attention should be paid to the following points:

(1) Staining instructions must be followed accurately.
(2) Staining solutions should be filtered before use, unless the solvent is volatile, in which case the fluid should be carefully decanted.
(3) Some staining solutions have to "ripen" (e.g. haematoxylin), others must be made up immediately before use (e.g. iron haematoxylin).
(4) Sections must be covered evenly by staining solution.
(5) If the stain solvent is volatile, the preparation should be covered (e.g. with watchglass, or beaker lined with damp filter paper) during staining: otherwise the solvent tends to evaporate, and the stain is precipitated on the preparation.
(6) Staining solutions must not be diluted (except in special cases, e.g. Leishman's stain).
(7) Staining can be intensified by warming to 37°C, or by increasing the length of time for staining.

DETAILS OF CERTAIN METHODS OF STAINING

Staining of tissues can be carried out either in bulk, or (more usually) on sections.

A. Staining in Bulk

This method is of value when large quantities of material are being dealt with, and where general information is required rather than minute detail. It is particularly useful for investigating the nervous system, for small embryos, and for dealing with sections for a large class.

(1) Haematoxylin and Eosin

Fix in a dichromate mixture: wash in running water for twenty-four hours.

Transfer to 25 per cent alcohol for twelve hours, and then 50 per cent alcohol for twenty-four hours. Transfer to

Scott's haematoxylin, 1 part
Acetic acid 2 per cent, 5 parts

for any time up to three weeks: penetration depends on consistency of tissue.
Wash in running tap water for twenty-four hours.
Transfer to 0·5 per cent aqueous eosin for twenty-four hours.
Take up through the alcohols, each containing eosin in solution, until absolute alcohol is reached.
Dehydrate, clear in xylol, and embed in wax.

(2) Orcein

Fix in a dichromate mixture: wash in running water for twenty-four hours.
Transfer to

Orcein	1 gm.
Hydrochloric acid (conc.)	1 ml.
90 per cent alcohol	100 ml.

for one week.
Transfer to acid alcohol for two days to differentiate. Wash quickly in distilled water.
Dehydrate, clear, and embed in wax. If overstained, sections can be further differentiated.
Sections must be floated on distilled water, not tap water (which tends to be alkaline).

(3) Carmalum

Fix material in mercuric acetic or Susa for preference.
Wash well in water (overnight).
Stain in carmalum, diluted 1/100 with 5 per cent potassium alum, until intensely coloured. This will take about 7–14 days.
Rinse in 5 per cent potassium alum for several hours.
Rinse in water for several hours.
Dehydrate in alcohols, clear in xylol, embed in wax and section.

B. STAINING OF SECTIONS, SMEARS, Etc.

(1) General Staining with Haematoxylin and Eosin

Take the section to 25 per cent alcohol.
Stain in filtered Scott's haematoxylin for three to eight minutes.
Wash in *alkaline* tap water or very dilute ammonia solution, many changes, for ten minutes or longer.
Remove excess water. Counterstain in filtered 1 per cent aqueous eosin for one minute.
Wash in tap water for one minute.
Take up through ascending strengths of alcohol.
Dehydrate, clear and mount in a synthetic resin.
(Nuclei blue. Cytoplasm pink. Blood orange. Connective tissue and muscle pink.)

(2) General Staining with Haematal 8 and Biebrich Scarlet

This technique, devised by J. R. Baker (*Quart. J. Micr. Sci.*, **103**, 493, 1962), is most highly recommended for general use. It is quick, rational, the reagents are ready for use immediately they are mixed and no differentiation is required.

Bring sections to water.

Stain in Haematal 8 solution (see Section E) progressively; this will usually be about 10 minutes.
Wash in running water 3 minutes.
Rinse in distilled water.
Counterstain in Biebrich Scarlet ½ minute.
Rinse in distilled water.
Dehydrate and pass through xylol before mounting in a synthetic resin.
Chromatin and strongly basiphil substances—blue; acidophil substances—pink.

(3) General Staining with Iron Haematoxylin and Van Gieson's Stain

Take the section to 25 per cent alcohol.
Stain in freshly made iron haematoxylin for six to eight minutes.
Wash in *alkaline* tap water, many changes, for ten minutes or longer.
Remove excess water. Counterstain in van Gieson's stain for one minute.
Wash in 96 per cent alcohol (*not* water, which removes the counterstain).
Dehydrate, clear and mount in synthetic resin.
(Nuclei dark brown. Collagen pinky-red. Elastin yellow. Muscle orange.)

This technique is extremely valuable for surveying the distribution of connective tissue in an organ.

(4) Staining with Orcein for Elastic Fibres

Take sections to 96 per cent alcohol.
Stain in orcein for an hour or more. (Keep covered to prevent evaporation.)
Wash in 96 per cent alcohol and examine.
If overstained, differentiate in 75 per cent alcohol, or acid alcohol. (The stain comes out very readily.)
Dehydrate, clear and mount in synthetic resin.
(Elastic fibres and granules dark red. All else *very* faintly stained, if at all.)

(5) Staining with Weigert's Resorcin-fuchsin for Elastic Fibres

Take sections to 96 per cent alcohol.
Stain in Weigert's stain for *at least* an hour, in well-stoppered jar.
Rinse in 96 per cent alcohol, until sufficiently differentiated.
Take quickly through 70 per cent alcohol to water, and counterstain for ten minutes or more in safranin (1 per cent aqueous).
Rinse in water, transfer to 96 per cent alcohol.
Dehydrate, clear in xylol, mount in synthetic resin.
(Nuclei red. Elastin fibres dark blue or black.)

6) Staining of Areolar Tissue

Make a film (or stretch) preparation on a slide: let it dry to the slide round the edges to keep it stretched, and keep it moist in the middle by breathing on it.

(i) **General Structure.** Fix in 96 per cent alcohol for ten minutes.
Stain with iron haematoxylin and van Gieson's stain, or with Haematal 8 and Biebrich Scarlet (see above).
Mount finally in synthetic resin.

(ii) **Elastic Fibres.** Fix in 96 per cent alcohol for ten minutes.
Stain with orcein (see above).
Mount finally in synthetic resin.

(iii) **Mast Cells.** Fix spread preparations in 96 per cent alcohol for ten minutes.
Stain with toluidine blue for five minutes.
Wash in 96 per cent alcohol.
If overstained, differentiate in acid alcohol.
Dehydrate and mount finally in synthetic resin.
(Mast cell granules dark purple-blue: cytoplasm pale greeny-blue: nuclei pale blue.)

(iv) **Fat.** Fix in 10 per cent formalin in 0·9 per cent NaCl for ten minutes.
Stain in Sudan III or Sudan black, keeping well covered to prevent evaporation, for twenty minutes.
Wash in water.
Stain in filtered haematoxylin for two minutes.
Wash in tap water for ten minutes.
Mount finally in an aqueous mountant or Farrants solution.
(Fat globules red or blue-black.)

(v) **Interstitial Substance.** Wash in 0·75 per cent Na_2SO_4.
Treat with 1 per cent $AgNO_3$ in the dark for five minutes.
Wash in distilled water.
Reduce either in sunlight (in water made slightly acid with acetic acid) or in 1 per cent metol.
Dehydrate, clear and mount in synthetic resin.

(7) Staining of Blood

Films of fresh blood are used. Slides must be free of alkali and of grease. Films must be spread evenly and thinly.

(i) Eosin and Methylene Blue

Fix the dried film by passing the slide three times through a small flame, film surface uppermost.
Stain in filtered alcoholic eosin (0·5%) for three minutes.
Wash well in tap water.
Blot lightly.
Counterstain in filtered 1 per cent methylene blue for one minute.
Wash well in tap water.
Blot lightly.
Dry in air.
When quite dry mount in synthetic resin.

(ii) Leishman's Stain

Cover the dried film with Leishman's stain and leave for one minute.
Dilute the stain on the slide with *distilled* water and leave staining for ten minutes or longer.
Wash well in distilled water.
Blot lightly.
Dry in air.
When quite dry mount in synthetic resin.

(iii) Giemsa's Stain

Fix the dried film in absolute alcohol for five minutes. Dilute the stain (1 drop of stain to 1 ml. of distilled water). Stain for thirty minutes.
Rinse in distilled water.

Blot.
Dry in air.
When quite dry mount in synthetic resin.

If stained blood films are to be examined only with an oil immersion objective, then they need not be mounted; in this case, the oil is applied directly to the stained film and removed after examination by dipping the slide in xylol.

(8) Staining of Bone Marrow

Make a film of marrow on a slide. Dry in air, or fix while still wet.
Fix in a mixture of equal parts of absolute alcohol and ether for ten minutes (this dissolves out the fat and fixes the cells). Use plenty of fluid and several changes.
Stain, as for a blood film, with alcoholic eosin and methylene blue, or with Leishman's stain.

(9) Staining of Nerve Fibres

(i) **Fresh.** Tease out a mixed nerve, keeping moist by breathing on it.
Fix in 96 per cent alcohol for ten minutes.
Stain with haematoxylin and eosin, or with iron haematoxylin and van Gieson's stain.
Mount finally in synthetic resin.
Alternatively, the nerve can be teased out in 1 per cent osmium tetroxide solution, and after staining for thirty minutes, mounted in Farrants medium or glycerol.

(ii) **In Sections of the Central Nervous System**

(*a*) *Myelin Sheath.* The myelin sheath can be stained differentially blue-black by the Weigert-Pal method. The principle of this method is that everything is overstained with haematoxylin by using double mordants, and the differentiation is carried out by oxidising the haematoxylin to soluble colourless substances until only the myelin still remains coloured.

Degenerating Myelin is stained by the Marchi method. Myelin, when breaking down, gives rise to an excess of very unsaturated fatty acids. When treated with $K_2Cr_2O_7$ the unsaturated fat of healthy myelin is easily saturated, but that of degenerating myelin is not. Subsequent treatment with osmium tetroxide will then blacken degenerating fibres (by production of the black lower oxide, OsO_2) but will not affect healthy fibres.

For details of these methods, a textbook of microtechnique should be consulted.

(*b*) *Axon.* This is best demonstrated by Ranson's $AgNO_3$ method. Fix in absolute alcohol + 1 per cent ammonia for forty-eight hours and rinse in distilled water. [*Or*, fix in formalin and wash in running tap water at least twenty-four hours.]
Transfer to pyridine for twenty-four hours.
Wash in repeated changes of distilled water until all smell of pyridine has gone (at least twenty-four hours).
Transfer to 2 per cent $AgNO_3$ in the dark at 35°C for one to three days.
Rinse in distilled water.
Reduce in pyrogallol (4 gms. in 100 ml. of 5 per cent formalin, made up in distilled water) for about five hours in the dark.
Wash. Dehydrate. Clear. Embed. Cut at 10 μm. Dewax and mount in synthetic resin.

(c) *Neurofibrils, etc.* These are best demonstrated by Bielschowsky's $AgNO_3$ method.

For details, see a textbook of microtechnique.

(10) Staining of Nerve Endings

(i) **Fresh.** Vital staining with methylene blue gives good results that are not easily rendered permanent.

Remove the tissue with as little damage as possible: damaged tissue stains intensely.
Cover with 0·05 per cent methylene blue in isotonic saline for thirty minutes.
Examine in isotonic saline.
This stain fades on covering up, as the living tissues reduce the methylene blue to the colourless base.
Permanent preparations may sometimes be achieved by transferring to saturated aqueous ammonium picrate, ½ to 1 hour.
Then put into a mixture of ammonium molybdate 1 gm.
distilled water 20 ml.
hydrochloric acid 1 drop
for one hour or more.
Wash quickly in distilled water.
Dehydrate very rapidly in alcohols.
Clear in xylol. Mount in synthetic resin.

Impregnation with gold chloride also gives good results, and these preparations are permanent. (Metal instruments must not be used.)

Treat the fresh tissue with 25 per cent formic acid for ten minutes.
Transfer to 1 per cent $AuCl_3$ in the dark at 37°C for about an hour.
Put back into 25 per cent formic acid for twenty minutes.
Transfer to pure glycerol.
Tease and examine in glycerol.

(ii) **In Sections.** Impregnation with $AgNO_3$ gives the best results. Ranson's method (see p. 441) nearly always succeeds.

C. SOME HISTOCHEMICAL METHODS

(1) **The Feulgen Method for DNA** (Deoxyribose nucleic acid).

Fix in formol saline or Helly's fluid. (Zenker's fluid in which an equivalent volume of 40 per cent formaldehyde is substituted for glacial acetic acid).
Embed in paraffin wax.
Cut paraffin sections at 5 μm. and bring to distilled water, treating with iodine (p. 433).
Hydrolyze in N HCl 5–7 mins. at 60°C.
Quick rinse in distilled water.
Schiff's reagent 30–45 mins.
Wash in running tap water 2–3 mins.
Counterstain in aqueous light green as desired.
Dehydrate, clear in xylol, mount in synthetic resin.
DNA, magenta; Cytoplasm, green.
A control experiment should be carried out in which the DNA is removed by extracting the section for 15 minutes with 4 per cent aqueous trichloracetic acid at 90°C.

(2) **The Periodic Acid—Schiff (PAS) Technique for Glycogen and other Polysaccharides and Mucoproteins containing Vicinal-glycol Groups.**

Fix in 10 per cent neutral formol-saline or in Bouin's fluid (without acetic acid).
Embed in paraffin wax.
Cut 5 μm. paraffin sections and bring to water.
Oxidize in periodic acid 5–7 mins. at room temperature.
Schiff's reagent 15–45 mins.
Running tap water—5 minutes.
Counterstain with aqueous light green as desired.
Dehydrate, clear in xylol and mount in synthetic resin.
A control slide in which the tissue has been incubated in 1 per cent diastase solution for 1 hour at 37°C will enable a positive reaction due to glycogen to be distinguished.
All substances containing vic-glycols (e.g. goblet cell mucin, glycogen, some cartilage matrix etc.) react strongly and are coloured red/magenta.

(3) **Gomori's Method for Alkaline Phosphatase (Gomori, 1950).**

Fix *small* pieces of tissue in 80 per cent ethanol or 10 per cent neutral formalin at 4°C for 3–6 hrs. Alternatively, freeze-dried material may be used.
Dehydrate and embed in a low melting point paraffin wax as rapidly as is consistent with good penetration.
Cut sections at 5 μm. and flatten on to glass slides in the usual way, but avoiding a high temperature. Dry at 37°C and store at 4°C.
Bring sections rapidly down to distilled water.
Incubate at 37°C in Gomori's substrate for ½–2 hr.
Rinse *rapidly* in distilled water.
Place in 2 per cent cobalt nitrate for 2 minutes.
Rinse *rapidly* in distilled water.
Place in distilled water to which ammonium sulphide has been added (2 drops per 50 ml.). 1 minute.
Wash well in tap water.
Counterstain if desired in light green or neutral red.
Dehydrate and mount in synthetic resin.
Sites of enzyme activity are black due to deposits of cobalt sulphide.
To demonstrate the absence of preformed phosphate some sections should be incubated in the substrate *without* the addition of calcium chloride.
As a control, the enzyme itself may be inactivated by heat or by the inclusion of sodium fluoride in the incubation medium.

(4) **Pyronin and Malachite Green (P/MG) for the differentiation of DNA and RNA** (Baker and Williams, *Quart. J. Micr. Sci.*, **106**, 3–13, 1965).

Fix small pieces of tissue in Zenker's fluid for 3 hours.
Embed in paraffin wax and section at 5 μm.
Bring sections to water, removing mercury precipitate by iodine treatment.
Stain in P/MG for 20 mins.
Blot dry and dip momentarily in a 0·025 per cent solution of sodium bicarbonate.
Dip in 96 per cent alcohol.
Absolute alcohol, two changes, for a total time of exactly 2 mins.
Xylol, mount in synthetic resin.
RNA—red; DNA—blue/green.
This technique is not a strict histochemical test unless a RNAase control extraction is carried out.

Take section to water and carefully blot.
Cover with a few drops of 0·05 per cent crystalline ribonuclease solution in distilled water.
Incubate at 37°C for 30–90 minutes in a covered Petri dish kept humid with damp filter paper.
Wash off enzyme and stain with P/MG.
RNA staining will have disappeared or been greatly reduced; nuclei will still stain green.

D. INJECTION METHODS

Systems of tubes may be rendered more easily recognizable in microscopical preparations by filling them with some coloured substance. The fluid originally present must first be displaced (if possible) by a suitable isotonic fluid at suitable pressure and temperature.

(i) Cold Injection

(*a*) Indian ink is easy to inject and remains in the vessels reasonably well if the tissue is fixed in 96 per cent alcohol.
(*b*) Milk can be injected and the tissues fixed and hardened in 7 per cent formalin containing 1 per cent of acetic acid. Frozen sections stained with Sudan III show the vessels containing red-stained fat.

(ii) Warm Injection

The animal (after death) must be kept warm and the injection carried out in a bath of warm saline. The best injection mass to use is gelatin coloured with carmine, which is liquid at the temperature used. Subsequent fixation in cold 96 per cent alcohol hardens the mass *in situ*, and paraffin sections can be prepared in the usual way.

(iii) Phagocytic Colouring

A harmless pigment (e.g. trypan blue in isotonic solution) is introduced into the circulation of the living animal and accumulates in some tissues of the R.E. system.

The pigment particles phagocytosed by the R.E. cells may be visualized by fixing the tissue and embedding in paraffin wax. Subsequent sectioning, followed by staining with a dye of contrasting colour to that injected allows the cells which show dye particle uptake to be easily seen.

E. FORMULAE OF SOLUTIONS MENTIONED IN THE APPENDIX

Acetate Buffer

For Pyronin and Malachite green

0·2 N acetic acid	81 ml.
0·2 M sodium acetate	119 ml.

Acid Alcohol (for differentiation of stains)

Alcohol, 96 per cent	70 ml.
Distilled water	30 ml.
Hydrochloric acid conc.	0·5–1 ml.

Biebrich Scarlet

0·1 per cent aqueous solution

Bouin's Fluid

Saturated solution of picric acid	75 ml.
Formalin	25 ml.
Glacial acetic acid	5 ml.

Carmalum

Potassium alum	5 gm.
Carmine	2 gm.
Water	100 ml.

Mix and boil together for 1 hour; cool and filter.

DPX Synthetic Mounting Resin Medium

This is a medium composed of distrene, a plasticizer and xylol. It is best bought ready-made.

Ebner's Fluid

Concentrated hydrochloric acid	5 ml.
Sodium sulphate	5 gm.
Alcohol, 96 per cent	500 ml.
Distilled water	1,000 ml.

E.D.T.A. (Ethylene-diamine-tetra-acetic Acid) for Decalcification

A solution of 10 per cent E.D.T.A. (sodium salt), adjusted to pH 6·5–7·5 with NaOH may be used. The time required for decalcification varies according to the size of the tissue but on average would be from 7–14 days.

Eosin

Aqueous

Eosin (water soluble)	0·5 gm.
Distilled water	100 ml.

Alcoholic

Eosin (alcohol soluble)	0·5 gm.
Alcohol, 96 per cent	100 ml.

Farrants Medium

Consists of glycerol and gum arabic, together with a preservative.
It is best bought ready made.

Giemsa Stain

Giemsa stain powder	1 gm.
Methanol	50 ml.
Glycerol	50 ml.

Triturate together in a mortar and pestle, adding alcohol and glycerol in small amounts over a period of 15 mins. Transfer to a stoppered bottle and allow to stand for 24 hours, shaking at intervals. Filter.

Glycerol Jelly (Baker)

Gelatin	5 gm.
Glycerol	35 ml.
Cresol	0·25 ml.
Water	65 ml.

Soak the gelatin in 25 ml. of the water for 1 hour then melt at 60°C. Mix the glycerol with the 40 ml. of water and add the cresol; heat to 60°C and mix with the gelatin.

Gomori's Substrate for Alkaline Phosphatase (pH circa 9·0)

3 per cent sodium glycerophosphate	10 ml.
Sodium barbitone	1 gm.
2 per cent calcium chloride	5 ml.
10 per cent magnesium sulphate	1 ml.
Distilled water	to make 50 ml.

Haematoxylin (Scott)

Haematoxylin	1·25 gm.
Glycerol	100 ml.
Distilled water	100 ml.
Alcohol, 96 per cent	100 ml.
Glacial acetic acid	10 ml.
Potash alum	7 gm.

Dissolve the haematoxylin in some of the water: add the glycerol and mix. Add the alum (dissolved by heating in the rest of the water). Lastly add the alcohol and the acid.

Allow to ripen 2–3 months.

Haematal 8

Stock (A)	Aluminium sulphate 16 H_2O	15·76 gm.
	Water	1 litre
Stock (B)	Haematein	1·876 gm.
	Ethylene glycol 50 per cent v/v aq.	1 litre

The dye mixture is prepared by adding an equal volume of stock solution A to stock solution B. It is ready for immediate use.

Iron Haematoxylin

Soln. A.	Haematoxylin	1 gm.
	Alcohol, 95 per cent	100 ml.
Soln. B.	Ferric chloride	0·6 gm.
	Distilled water	100 ml.
	Hydrochloric acid concentrated	1 drop
Just before use mix—		
	Solution A	50 ml.
	Solution B	50 ml.

Leishman's Stain

0·15 gm. Leishman's stain.

Triturate with 100 ml. methyl alcohol (acetone free).

Filter. Keep well stoppered.

Löffler's Methylene Blue

Methylene blue, saturated solution in absolute alcohol	30 ml.
Potassium hydroxide, 0·01 per cent, in water	100 ml.

Marchi's Fluid

Müller's fluid	20 ml.
Osmium tetroxide solution, 1 per cent	10 ml.

Mercuric Acetic

Mercuric chloride, saturated aqueous solution	100 ml.
Glacial acetic acid	2 ml.

Methylene Blue (for blood films)

Methylene blue	1 gm.
Distilled water	100 ml.

Müller's Fluid

Potassium dichromate	2·5 gm.
Sodium sulphate	1 gm.
Distilled water	100 ml.

Nitric Acid for Decalcification

Formalin, 10 per cent	100 ml.
Nitric acid, conc.	15 ml.

Use a large volume with respect to the tissue and change once a day. Tissues will require up to a week to decalcify.

Orcein

Orcein	1 gm.
Alcohol, 96 per cent	100 ml.
Hydrochloric acid concentrated	1 ml.

Periodic Acid (for PAS technique)

Periodic acid	1 gm.
Distilled water	100 ml.

Phenol Xylol (carbol xylol)

Phenol, pure white crystals	100 gm.
Xylol	300 ml.

Pyronin/Malachite Green

Pyronin Y, 4 per cent aqueous	40 ml.
Malachite green 0·3 per cent aqueous	10 ml.
Buffer solution, pH 4·8 (see p. 435)	50 ml.

NOTE: Malachite Green has been substituted by Baker for the methyl green of his original technique in order to avoid the need for extracting the methyl violet which occurs as a contaminant of methyl green.

Schiff's Reagent (for Feulgen and PAS)

Add *very slowly* 1 gm. of basic fuchsin to 400 ml. boiling distilled water. Shake for 5 mins.
Cool to 50°C, filter and add 1 ml. thionyl chloride ($SOCl_2$).
Leave in cool, dark place overnight.
Decolorize by adding 2 gm. powdered animal charcoal and shake for 1 min.
Filter before use.
Store in dark, preferably at 4°C.

Sudan III

Alcohol, 70 per cent	50 ml.
Acetone	50 ml.

Mixture saturated with Sudan III, filtered, and kept well stoppered.

Sudan Black B

Alcohol, 70 per cent saturated with Sudan black B, by refluxing for 20 mins. Filter before use.

Susa

Distilled water saturated with mercuric chloride	50 ml.
Trichloracetic acid	2 gm.
Formalin	20 ml.
Acetic acid (glacial)	4 ml.
Distilled water	30 ml.

Toluidine Blue

Aqueous	
Toluidine blue	1 gm.
Distilled water	100 ml.
Alcoholic	
Toluidine blue	0·5 gm.
Alcohol, 75 per cent	100 ml.

Trichloracetic Acid Decalcifier

Trichloracetic acid, 5 per cent	90 ml.
Formalin	10 ml.

Van Gieson's Stain

Picric acid, saturated aqueous solution	150 ml.
Acid fuchsin	0·2 gm.

Weigert's Elastin Stain (Resorcin Fuchsin)

Resorcin fuchsin stain (Gurr)	1 gm.
Alcohol, 96 per cent	100 ml.

Warm in water bath, with neck of flask lightly plugged, for twenty minutes. Cool. Filter. Add 2 ml. hydrochloric acid.

Zenker's Fluid

Potassium dichromate	20 gm.
Sodium sulphate	10 gm.
Mercuric chloride	50 gm.
Water	960 ml.

Add 50 ml. glacial acetic acid just before use

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