LIVING TISSUES

An Introduction to Functional Histology

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Preface

UNDERSTANDING of living tissues must often be gained through a study of dead ones, and structure cannot be considered apart from function. This book has been written as a brief introduction to the study of tissues, based in the first place on classical histological techniques, but extended to introduce ancillary methods of study, which can give functional significance to structural features. It is not a textbook of histology, and is not intended to be used as one. Only a few tissues have been considered in detail, and these have been chosen to illustrate how various techniques may be applied to their study. Little attention has been paid to electron microscopy, although this plays a major part in structural studies at the present time; this technique is considered more fully in other publications. It is hoped that this book may serve as an introduction to fuller texts, and a selected number of these are listed in the References.

Introduction

TISSUES of all animals consist of cells and intercellular material. Some, such as liver, are predominantly cellular; while others, such as bone, are composed mainly of intercellular material. Even the most cellular tissues, however, contain a certain amount of connective tissue; and all connective tissue contains cells.

Tissues may consist mainly of cells of a single type: liver is again an example of this; but all are more or less complex, and even the simplest are not only built up of cells and connective tissues in varying proportions, but also contain formed structures such as blood vessels, lymphatics and nerves, which form an integral part of the tissue, and are essential for its function. These components are in turn made up of various types of cells and connective tissue. Almost any sample of tissue removed from an animal will thus contain many different types of cell, although one type may, and often does, predominate.

Connective tissue is also very variable, both in its cellular content, and in its organization, which reflects its particular function. It may have a well-defined regular structure, as bone or tendon; or it may have no very characteristic organization, as the loose connective tissue surrounding organs such as the kidneys. Often, as already pointed out, it simply forms a part of some predominantly cellular tissue; and although it may in such a case be relatively inconspicuous, it nevertheless plays an essential role in the functioning of such tissue.

Progress in the branch of anatomy concerned with the study of the microscopic structure of tissues, known as histology, depended almost entirely on the development of the optical

microscope, and of techniques enabling thin sections of biological material to be cut and stained. Great advances in this field followed the introduction of the aniline dyes and their application to the staining of biological specimens, which occurred during the latter part of the nineteenth and the earlier part of the twentieth centuries. This period was associated with widespread interest in the microscopic structure of both botanical and zoological material. It was found that cells of various types could be readily distinguished on account of their affinities for different dyes; but since the reason why cells of one type stained with one dye, and those of another type with a different one was usually unknown, there accumulated a mass of purely descriptive data on the structure of tissues which often had little bearing on the functional significance of such structure.

Even as early as the middle of the nineteenth century, however, a few biologists were interested in the chemical characteristics of tissues, and attempted to show the presence of particular substances in cells by means of staining reactions; staining of starch by iodine was an early example of this kind of study. At first interest was directed mainly towards botanical material; and since many of the methods used at this time to reveal chemical compounds were relatively crude and resulted in destruction of the tissue, it was often difficult to correlate chemical characteristics with particular structural features. Nevertheless this early work eventually led to the development of a new approach to histology, the study of the chemistry of tissues, or histochemistry.

Histochemistry is still a relatively undeveloped subject, and as yet we can only demonstrate a relatively small proportion of the chemical substances which occur in tissues; furthermore, the functional significance of many of the substances which can be demonstrated is often not clear. Histochemistry is, nevertheless, particularly valuable as a link between purely structural and biochemical studies. Biochemistry can tell us much about the chemical reactions which take place in tissues; but the technical methods used in biochemistry often make it difficult to localize these reactions very closely. For example, many biochemical

studies are made using either slices of tissue, or homogenates in which the samples are reduced to a pulp before chemical analysis. The chemical composition of such preparations can be determined, and the concentrations of active substances such as enzymes estimated; but the final picture is one of the chemistry of the whole tissue; and since as already pointed out even relatively simple tissues are not made up of cells of a single type, this gives us little idea of the distribution of chemical activity within the sample. Histochemical techniques can supplement such studies by revealing the distribution of chemical substances in relation to the structural components of the tissues; and we now know that cells which all appear similar when prepared by ordinary histological methods may in fact differ considerably from each other in their chemical make-up, and in their activity at any given time. Histochemistry may thus show regional differences in activity which would not otherwise be apparent, and in this way it may act as a link between biochemical and morphological studies.

Tissue to be examined with the optical microscope must usually be "fixed" by immersion in a fixative (see p. 1) and cut into thin sections, which are then stained to produce contrast between various structures in the specimen. This has often led to difficulties in interpretation of what is seen in these fixed and stained sections, and has given rise to argument as to whether certain structures which can be observed with the microscope are actually present during life, or whether they are artifacts produced during the preparation of the tissue for examination. This difficulty can be resolved if structural features can be seen in living tissues as well as in fixed specimens; but the ordinary light microscope is not a very satisfactory instrument for such studies, since there is usually little contrast between the various components of the tissues when they are neither fixed nor stained. Relatively recently, however, methods of microscopy have been developed which increase the optical contrast of cells and of intracellular inclusions, and enable a great deal of detail to be distinguished in living or fresh untreated specimens. The

phase-contrast microscope, which is briefly described below, is the commonest of such instruments.

The scope of optical microscopy is also limited by virtue of the fact that the resolution, or the ability to distinguish as separate structures two points lying close together, is limited by the wavelength of the light used to illuminate the specimen. Useful magnification is thus also limited. In recent years the electron microscope has greatly extended the feasible range of studies of fine structure. This instrument uses a beam of electrons in place of light, and focussing is carried out by means of electromagnetic fields. Since the wavelength of the electron beam, although variable, is about 1/100,000 that of visible light, the resolution, and hence the useful magnification obtainable, is enormously greater than with the optical microscope. Morphological studies can indeed now be carried into the size range of molecules (see References).

No single method of study of tissues can give a complete picture of their structure and function. Ideas of microscopic structure are still largely based on the methods of classical histology; but these ideas can now be extended by data gained from other types of study. In the first section of this book, the classical methods of study of tissues are considered; subsequently the newer methods of study now available are reviewed, and it is shown how a combination of histology and histochemistry, studies of living tissue, electron microscopy and the use of experimental methods can enable us to arrive at some understanding of the relationship between structure and function.

CHAPTER I

The microscopic study of tissues

THE PREPARATION OF TISSUES FOR STUDY

As already noted in the introduction, most routine studies of tissues are still based on the methods of classical histology, and these have been supplemented rather than replaced by the more recently developed techniques such as histochemistry and new methods of microscopy. Examination of tissue by the optical microscope, however, usually involves the cutting of thin sections, which are then mounted and stained before examination. Since the various treatments to which the specimen is subjected before examination may considerably alter its appearance, it is appropriate to consider in outline the principles behind the various preparative techniques, and the effects which these procedures may have upon the structure of tissues.

FIXATION

As soon as the living processes of a tissue are grossly disturbed, either by death of the animal, or by removal of the tissue from the living animal, changes in structure begin. In some tissues more than in others, cutting off of the blood supply is followed within seconds by the onset of autolysis, which is literally "self-digestion". The various enzymes present in the cells, whose activity is controlled while the tissue is alive, after death begin to attack the structure of the cells in which they are contained. Destruction of finer features of the cells begins at once, and may

progress so far that many of the cellular characteristics are lost.

Other factors may also lead to changes in cellular structure. Drying of a piece of tissue will result in distortion of the cells; but on the other hand, if the tissue is placed in fluid which is not isotonic with that inside the cells, osmotic effects will cause either shrinkage or swelling, and once again an abnormal picture will be produced. A further hazard is that dead tissue is liable to be attacked by moulds or bacteria, which again will cause structural damage.

Some of these changes can be prevented, and others minimized by treatment of the tissue with a fixative. This is a mixture of chemicals, usually dissolved in water or some other solvent, which acts partly as a preservative and prevents autolysis and bacterial decay, but which also acts on the constituents of the tissue and modifies or actually combines with them chemically or physically. This is the process of fixation. Ideally a fixative should react with the tissues without causing any major structural changes, so that they retain a form similar to that of the living state; and also, by its reaction with the components of the tissues, it should protect them against any distorting effects of procedures undertaken later during the preparation of the specimen for examination with the microscope.

Inevitably even the best fixative mixtures produce some alteration in the microscopic appearance of the tissues, and it must always be remembered when examining histological specimens that some of the structural features seen under the microscope may not have been present during life, but may be artifacts produced by fixation. The full extent of such changes may not be easily appreciated unless it is possible to examine a sample of living tissue and compare it with fixed specimens, or even to watch the actual process of fixation under the microscope. In some cases however the artifacts may be so gross that they are obvious, For example, if a piece of liver is placed in Carnoy's fixative (a solution of alcohol, acetic acid and chloroform) the cytoplasmic contents of cells lying near the outside of the block of tissue may be displaced to one side of the cells by the penetrat-

ing wave of fixative; cells near the centre of the block, exposed more slowly and evenly to the penetrating solution, show no such displacement (Plate I, Fig. 1).

Protein is the most important component of animal tissues, and the main action of the majority of fixatives is on protein rather than on fat and carbohydrate. Fixatives can be divided, according to their action, into coagulant and non-coagulant types. The former precipitate protein, forming a coarse or fine meshwork; while the latter react with the protein, and considerably reduce its solubility without precipitating it. Salts of mercury act as precipitant fixatives, while formaldehyde is a good example of the non-precipitant type. Many fixatives used in biological work are mixtures of several different chemical substances, and many contain at least one precipitant. The chemistry of fixation is still by no means fully understood, but many aspects of the techniques have been considered by Baker (1958).

EMBEDDING

After a tissue has been fixed it must be infiltrated with some substance which will give it enough support to allow thin sections to be cut. These are often only a few thousandths of a millimetre (microns, often written μ) in thickness, and paraffin wax is the commonest medium used to give the necessary support. Before the tissue can be infiltrated with wax, however, all the water must be removed. This is usually achieved by passing the specimen by stages through a graded series of alcohols of increasing strength, up to absolute (100 per cent). Gradual dehydration produces less distortion of the tissue than if the specimen were placed directly from the water into absolute alcohol, which would effect a rapid removal of the water. After passing through absolute alcohol, the specimen is next treated with a wax solvent such as xylene, which replaces the alcohol, which would not mix with wax. Finally, the tissue is placed in a container of molten wax in an oven kept at a temperature few degrees higher than its melting point, which is usually about 56°C. After allowing enough time for complete infiltration, the

specimen is then embedded by placing it in a small container of molten wax, which is allowed to cool, so that a block of wax and wax-infiltrated tissue is formed which, after trimming, is ready for sectioning.

During dehydration and embedding the specimen is exposed to various solvents, and these may extract materials such as fat or lipid from the tissue. Such a method of preparation is therefore not suitable for studying the distribution of soluble materials, unless the sample has first been treated in some way to render these insoluble. Loss of such soluble material, and distortion due to shrinkage during the process of dehydration, are thus further examples of artifacts that may be introduced during the preparation of biological specimens for microscopic examination.

CUTTING AND MOUNTING SECTIONS

Thin sections are cut from wax-embedded material by means of an instrument called a microtome. There are various types of these; essentially they are machines which enable the operator to cut series of sections at a constant, though variable, thickness. The choice of thickness depends on the type of tissue to be studied, and on the aims of the study. Examination of the structure of individual cells is easiest in sections whose thickness is less than the diameter of the cells; in thicker sections layers of cells will overlie each other, and make it difficult to study fine detail. Thick sections (25 μ or more) are preferable for the study of the grouping of cells, as in the "nuclei" of the central nervous system. In such cases, the superimposition of several layers of cells will give a clearer picture of the arrangement of the various groups than would be possible in a thin section.

After the sections have been cut, they are mounted, still infiltrated with wax, on glass slides coated with a thin layer of albumin, which acts as an adhesive. Creases are removed by flooding the slides with water so that the sections float, and then warming on a hotplate until they flatten. Surplus water is then run off, the sections are positioned with a camel hair brush, and

the slides are dried either on a hotplate or in an oven. After drying, the sections will be firmly attached to the slides, and should remain so during the staining.

STAINING

Before the sections are stained, the wax which permeates them must be removed by placing the slide in a solvent such as xylene. The xylene is itself removed by rinsing the slide in absolute alcohol; and the treatment from then on depends on the method of staining to be used. Many common stains are dissolved in water; but some are made up in alcohol, which may be absolute or dilute. If an aqueous stain is to be used, then the slide is passed through a series of graded alcohols to water before being transferred to the stain; but if an alcoholic stain, then the slide need only be passed through graded alcohols to one of the same dilution as that in which the stain is dissolved.

A common method of staining uses two stains, haematoxylin, which is a basic dye, and stains nuclear chromatin, and eosin, a red dye which is used as a counterstain to colour those structures which have not taken up the basic dye. In practice, the sections are brought down to water, as described above, placed in a solution of haematoxylin, and left there until they are overstained. The excess stain is removed from the sections by rinsing the slide in a weakly acidic solution until the desired intensity is achieved; this process of "differentiation" is controlled by microscopic examination. The sections are washed thoroughly in running water, which "blues" them, restoring the blue colour of the haematoxylin which had been reddened by the acid. They are then counterstained with eosin, washed to remove excess stain, and dehydrated by passing again through a series of graded alcohols to absolute. After clearing in xylene, the sections are mounted by placing a drop of some medium, such as Canada balsam dissolved in xylene, on them, and covering with a thin glass cover-slip. The balsam infiltrates the sections, and since its refractive index is close to that of the tissue, the section is rendered highly transparent, so that only stained structures can be seen

and distinguished, either by their form, or by the intensity and colour of their staining.

More complex methods of colouring sections can be used, and they may be stained by several dyes one after the other; each stain, having particular affinity for some part of the tissue, will selectively colour this. Alternatively the sections may be immersed in a mixture containing two or more stains, so that the various tissue components will each take up the dye for which it has the strongest affinity.

EXAMINATION OF TISSUES

Optical microscopy

The ordinary optical microscope remains the most widely used and readily available instrument for the examination of tissues. The amount of detail revealed by this instrument depends largely on its resolving power and magnification, but in addition on the contrast between the various structural features in the specimen and the background. This contrast depends on variations in amplitude of the light waves passing through different parts of the object examined. In living tissues the range of such variations is relatively slight, and hence the optical microscope reveals little detail. Fixation and staining greatly increase the range of variation in amplitude, and make various components of the tissues readily visible and distinguishable from each other. Fixation, cutting of thin sections, and staining are thus essential procedures if the optical microscope is to be used to its greatest advantage in the study of the organization of tissues. As already noted, however, it is very likely that artifacts of various kinds are produced during the preparation of sections for microscopic examination, and one way in which the extent of such artifacts can be assessed is by the study of living or unfixed material. In recent years the phase contrast microscope has become widely available, and can be used for such studies.

PHASE CONTRAST MICROSCOPY

It has already been noted that most living cells are very

transparent. This means that there is little change in the intensity of light transmitted through them, and also little variation in the intensity of light transmitted through different parts of single cells. In effect, thin sections of most tissues are relatively colourless, except for such elements as cells filled with pigment, or red blood-cells with their contained haemoglobin. Hence there is little differential absorption or transmission of light of particular wavelengths, and the eye cannot detect structural features by means of changes in wavelength of transmitted light, which are apparent as colours in a stained section.

Living or unstained cells and tissues do, however, produce changes in the *phase* of the light-waves passing through them. This is because light passing through an optically dense medium is slowed with respect to light passing through a less dense one. The density of cytoplasm in various parts of a cell varies, and organelles such as mitochondria, or secretory granules, are optically denser than the surrounding cytoplasm. The phase contrast microscope is essentially a device for converting these small changes of phase into variations in intensity or amplitude, which can be detected by the eye. By this means such structures as nuclei, chromosomes, nuclear and cytoplasmic membranes and inclusions can be readily observed in living and unstained cells.

FLUORESCENCE MICROSCOPY

Fluorescence is the phenomenon of the emission of light by some material at a wavelength which is longer than that of the incident illumination. As applied to biological studies, specimens, usually in the form of thin sections, are illuminated either by ultraviolet light, which is invisible to the eye, or by light of a wavelength lying towards this end of the visible spectrum. Fluorescent materials in the specimen are then shown up by their emission of light whose wavelength falls within the visible spectrum, outside the blue–violet range.

Fluorescence microscopy can be used to demonstrate fluorescent material which is present as a normal constituent of cells, such as vitamin A or riboflavin; this is making use of the property

of "autofluorescence". Alternatively, sections can be stained with fluorescent dyes which, just as with ordinary dyes, often have a particular affinity for certain substances or structures in the tissues, which then become fluorescent on account of the dye bound to them. A stain called acridine orange, for example, can be used in this way to demonstrate nucleic acids. A further application of fluorescent staining depends on the fact that fluorescence is detectable even when only very low concentrations of materials are present. Because of this, fluorescent compounds can be used to stain living tissues, and will be visible when they are present in concentrations too small to have any toxic effects.

POLARIZATION MICROSCOPY

Material whose refractive index varies with the direction in which light is transmitted through them exhibit the property of birefringence. This may be due to regularity of molecular structure, or to uniform orientation of particles within some medium. In biological specimens, such orientation is found particularly in fibrous structures, in which there is often an orientation of molecules along the length of fibres; examples of this are found in chromosomes, and in muscle fibres. Such birefringence can be demonstrated by the polarization microscope.

Details of methods of optical microscopy such as those described, and others such as diffraction microscopy, can be found in Françon's textbook.

ELECTRON MICROSCOPY

The resolution of a microscope is limited by the wavelength of the light used to illuminate the object. Using visible light, the theoretical resolution is a little more than 0·25 μ. Decreasing the wavelength of the light, by using blue or ultraviolet illumination, will give greater resolution than using ordinary light; but the greatest advance in this respect has been the development of the electron microscope.

The principle of this instrument is that a beam of electrons is used in place of visible light. The wavelength of such a beam

varies with the applied accelerating voltage, but is in any event much smaller than that of light; hence the resolution, which varies with the wavelength of the illuminating beam, is much greater than that of the light microscope. Technical problems prevent the theoretical limits of resolution being reached, but in practice points 5 Å units apart can be distinguished with a high-resolution electron microscope in good condition. This is 1/2000 μ, compared with the maximum resolution of an optical microscope of about $\frac{1}{4}$ μ. (1 Å [Ångstrom] unit $= 1 \times 10^{-4}\mu$.)

The electron microscope uses magnetic fields in place of glass lenses. Since the beam of electrons has poor powers of penetration, it can only be transmitted through a high vacuum, and furthermore sections of the specimen to be examined must be extremely thin, a necessity which brings its own problems of embedding and sectioning. In addition, the beam of electrons is invisible to the naked eye, so that an image of the specimen cannot be seen directly, but only on a fluorescent screen, and detailed studies are made by means of photographs. High resolution and magnification will clearly magnify artifacts as well as normal structures, and fixative procedures must be carefully controlled for electron microscopy. Nevertheless, despite many technical difficulties, this instrument has extended the range of studies of the fine structure of cells and tissues, and provided some basis for the analysis of the relationship between structure and function at a submicroscopic level.

THE INTERPRETATION OF WHAT IS SEEN UNDER THE MICROSCOPE

Most studies of the microscopic structure of tissues involve the use of relatively thin sections. Inevitably this often gives rise to difficulties in interpretation, since it is not easy to relate the appearance of things seen in the slide to their actual three-dimensional form in the intact tissue. Furthermore, different planes of section through an asymmetrical structure will give entirely different ideas of its form.

As a simple example, consider the appearance of a medium

sized artery, such as the radial of the human forearm. This is essentially a muscular tube, lined by flattened endothelial cells lying on a layer of connective tissue, and covered on the outside with connective tissue which blends with that of the intermuscular septa in which the vessel lies. Sections cut transversely show the vessel simply as a round tube with a wall made up of three well-defined layers. Sections cut longitudinally near the mid-line will show an elongated tube with parallel sides; while sections cut across the vessel in some plane intermediate between transverse and longitudinal will show three-layered walls bounding a more or less ovoid central lumen.

Interpretation of sections such as these is fairly straightforward, provided that they include all three layers of the wall and the central lumen. Sections which pass through the tissue of the wall alone, without showing a central lumen, are more difficult to recognize. In this event the structure must be identified by means of the disposition of its component cells and connective elements relative to each other, and this involves an understanding of the intrinsic arrangement of the elements which make up the vessel. The middle layer of the wall of an artery such as the radial is made up largely of smooth muscle cells arranged concentrically around the lumen. The cells will be cut along their length, therefore, in transverse sections across the vessel, while sections cut tangentially to the wall will include smooth muscle cells showing a variety of profiles (Plate I, Figs. 2 and 3).

In some cases difficulties in interpretation of the organization of tissues can best be overcome by looking at sections cut in various planes through the tissue, and assembling a composite picture from these. In other cases a different kind of preparation may be helpful, such as a whole mount of the tissue in question. For example, the lining of the serous cavities of the body consists of a single layer of thin flattened cells with very little cytoplasm lying on a basement membrane of connective tissue. Transverse sections across one of these membranes, such as the pleura or peritoneum, give very little idea of the relationship of the lining mesothelial cells to each other. The individual cells indeed appear

as little more than nuclei projecting slightly from the surface of the tissue, and the cytoplasm of the cells is so scanty as to be almost invisible. If a piece of the serous membrane is spread out on a slide, and treated with silver nitrate to show up the boundaries between the cells, it can be seen that the mesothelium forms a "pavement" covering the whole surface of the membrane (Fig. 1). It would be difficult to arrive at such a picture from a study of sections cut vertically to the surface, although electron micrographs reveal the close relationship between the cells.

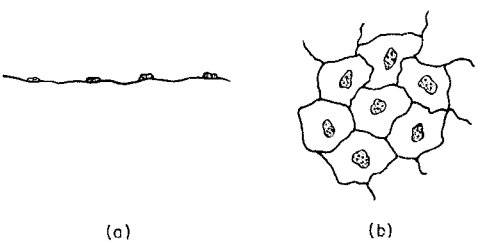


FIG. 1. Mesothelial cells. (a) in cross-section, (b) viewed from the surface.

Skin offers another example of how several different kinds of preparation can help in understanding the organization of a tissue in a way in which the study of thin sections cannot readily do. Skin is made up of a superficial cellular layer, the epidermis, and underlying layers of connective tissue which constitute the dermis. The junction of the epidermis and the dermis is irregular (Plate I, Fig. 4). In pigmented skin numerous cells filled with pigment lie along this junction and granules of pigment often appear to be lying between cells of the lower layers of the epidermis. The relationship of the pigmented cells to the epidermis is, however, difficult to appreciate in sections, but can readily be understood from an examination of a modified "whole mount" type of preparation, called "split skin".

The technique of preparing split skin involves cutting thin shavings from the skin with a sharp knife. Such shavings should include all the epidermis, but only the superficial layers of the

dermis. These fragments are then incubated in a solution of the enzyme trypsin, which digests the protein collagen fibres which bind the epidermis to the dermis. After about fifteen minutes digestion the sheet of epidermis can be lifted away from the dermis, and either laid with its outer surface down on a glass slide for immediate examination, or fixed, dehydrated and mounted as a permanent preparation.

Such a preparation gives a three-dimensional picture of the deep surface of the epidermis, and it can be seen that this surface has a pronounced "hill and valley" pattern, with depressions where papillae of dermal connective tissue penetrate into the epidermis. These irregular "pegs" of dermal connective tissue are responsible for the irregular line of the dermo-epidermal junction seen in sections.

The split skin preparation also reveals that the pigment-containing cells lie on the lower surface of the epidermis. When seen over their whole extent, these cells are found to consist of branching processes extending out from the perinuclear cytoplasm. These processes end in small expansions closely applied to the lower epidermal cells. It can be shown that these branching pigment cells, which have been called dendritic cells, are responsible for the elaboration of the pigment melanin, and the enzymes which take part in the synthesis can be demonstrated histochemically. The ordinary epidermal cells contain granules of melanin, but are unable to manufacture it, and the pigment is apparently injected directly into them from the processes of the dendritic cells.

Sections through skin in turn show a feature which is not easily seen in a whole mount preparation of epidermis: that the granules of pigment form a cap over the superficial aspect of the nucleus, where they are in a position to act as a protective shield against harmful ultraviolet radiation (Plate I, Fig. 4). The granules of pigment which in sections appear to lie free between the epidermal cells are in fact contained within the processes of the dendritic cells.

Many tissues do not lend themselves so readily to a three-

dimensional study, and more laborious methods must be used to build up a solid representation of a structure. One such method is to make a reconstruction of the specimen. To do this, serial sections are cut through the specimen, and sections, selected at regular intervals, projected at a suitable magnification on to paper, or alternatively on to thin sheets of wax or perspex. The specimen can then either be reconstructed graphically; or a solid model in wax or perspex can be made by cutting round the outlines of the various sections, and assembling the cut-outs to form a solid model. In order that this should be accurate, particular attention must be paid to the relationship between the thickness of the plates, and the real intervals between the chosen sections.

HISTOCHEMISTRY

The relationship between histology and histochemistry was briefly described in the Introduction. In this section the main types of histochemical techniques will be considered, with examples of their application to particular tissues.

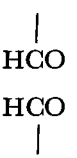
Broadly speaking, histochemical techniques fall into two main classes: those used to demonstrate substances present in relatively large amounts, which can then be directly examined under the microscope; and those used to demonstrate substances which are present in only small amounts, but which are chemically highly active, and are revealed by virtue of this activity. Techniques of the first class may be used to reveal inorganic or organic material; those of the second are most often directed towards the study of enzymes.

Histochemical demonstration of either inorganic or organic substances in cells and tissues may make use of well-known chemical reactions, but these are made to take place within the tissues concerned, and give reaction products which can be easily seen. Inorganic iron, for example, can be revealed by means of a Prussian blue reaction; sections of the tissue, suitably fixed, are immersed in an acid solution of potassium ferrocyanide. Any ferric ions in the tissue will then react with the ferrocyanide to

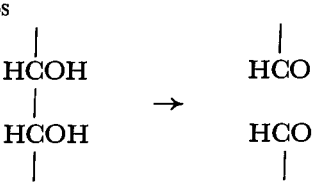
form a deep blue insoluable precipitate, which indicates the sites where iron is present (Plate II, Fig. 5).

Techniques used to reveal organic material are often more complex; but a very common one in histochemistry is that used to show the presence of carbohydrate in cells and tissue. This is the periodic acid-Schiff (PAS) reaction. It may be used to demonstrate pure carbohydrate such as glycogen (cf. Plate II, Figs. 6 and 7), but will also reveal more complex carbohydrate-containing substances, such as the glycoprotein hormones of the anterior pituitary gland.

The PAS technique is not a simple staining method, but depends on a chemical reaction being carried out on the carbohydrate material present in the tissue which renders it capable of combining with a decolourized dye to give a coloured end-product. The dye is basic fuchsin, which has been bleached by the action of sulphur dioxide to colourless leucofuchsin; this is called Schiff's reagent. By some reaction which is not entirely understood, this colourless product combines with molecules of organic material at sites where there are two adjacent aldehyde groups



Aldehyde groups so situated do not occur naturally in carbohydrate molecules, but are produced by the mild oxidation of 1 : 2 glycol groups



In practice this oxidation is brought about by exposing sections of tissue, mounted on slides, to a weak oxidizing agent, periodic acid. Stronger oxidizing agents, such as potassium

permanganate, are unsuitable, for these would continue the oxidation further to produce two ketonic (CO) groups, which do not give a coloured product with Schiff's reagent. As a result of the PAS reaction, carbohydrate material in tissues is revealed by its red or carmine staining.

As with other histochemical reactions, various controls are necessary to eliminate misleading or false-positive results due to non-carbohydrate materials. Distinction must also be made between various types of carbohydrate-containing substances; glycogen for example, which is pure carbohydrate, occurs in many different kinds of cells and can be differentiated from more complex substances such as the glycoprotein complexes in cells of the adenohypophysis. This differentiation is relatively easy, since glycogen is broken down by the enzyme diastase; sections treated with a solution of this enzyme (or with saliva, which also contains it) before being submitted to the PAS reaction will not show any glycogen; glycoproteins, however, remain unaffected, and are stained.

Many of the simple staining methods used in histology are empirical, so that although certain stains may have an affinity for particular components of cells and tissues, it is often not possible to relate this with any precision to the chemical make up, or to any specific chemical activity. Some dyes, however, do have affinities for particular classes of chemical substances, and provided that suitable controls are used, these properties can be applied to the demonstration of such materials.

Methyl green and pyronin are two dyes with such properties. The first of these has a particular affinity for desoxyribose nucleic acid (DNA), which is a major component of the chromosomes in the cell nucleus. The second, pyronin, has an affinity for ribose nucleic acid (RNA) which occurs both in the nucleolus and, in large amounts, in the cytoplasm of many cells which are in a phase of active synthesis of protein. Cells stained by a mixture of these two dyes will have greenish-blue nuclear chromatin, while the nucleoli and any cytoplasmic material containing RNA will be coloured red (Plate II, Fig. 8).

In this example, as in others, control procedures must be carried out, since it does not *necessarily* follow that only DNA and RNA will be stained by this mixture of dyes. Here, confirmation of the identity of the stained material can be obtained by treating control sections before staining with the enzymes desoxyribonuclease and ribonuclease, which break down DNA and RNA respectively. In a section treated with the first of these enzymes, there should be no staining with methyl green, if DNA is the only substance stained by that dye; similarly, the second enzyme should remove all material stainable by pyronin. Comparison of treated and untreated sections will thus enable the distribution of these two nucleic acids to be determined.

The three techniques described above are examples of histochemical methods which demonstrate some inorganic or organic substance which is present in cells in a relatively large amount, so that the actual substance in question is either stained by dyes, or takes part in a chemical reaction which results in the formation of a coloured end-product in which it is incorporated. A different kind of reaction must be used to demonstrate substances which, although highly active chemically, are often present in tissues in very small quantities. The outstanding class of substances of this kind is that including the enzymes. Enzymes can be subdivided into groups according to their activity, and are also usually identified with reference to this. Phosphatases, for example, bring about the hydrolysis of phosphate esters; dehydrogenases remove hydrogen; oxidases oxidize, and so on.

Many enzymes cannot yet be demonstrated histochemically, but methods for showing a considerable number of them have been developed. The techniques generally depend on the production of visible reaction products at the sites of enzymatic activity. The tissue, sometimes as a block, but more usually as sections, is incubated in a suitable substrate mixture containing a compound which is acted on by the enzyme in question. The recognition of the site of enzymatic activity depends on the precipitation of one of the products of this reaction at the site where it is formed. Such precipitation may occur spontaneously

if one of the products is sufficiently insoluble. If not, however, one of the products may be converted into an insoluble compound by interaction with some other constituent of the substrate mixture (see below). This insoluble compound may itself be readily visible under the microscope, but if it is not, it can be either stained, or converted into a coloured substance.

In all histochemical studies of enzymes, care must be taken to avoid a number of causes of inaccurate results. Firstly, the enzyme to be studied must not have been inactivated by any procedure during the preparation of the tissue; neither must it have diffused from the site which it normally occupies in the living tissue; and as already noted the reaction products, on which recognition of the site of activity depends, should be insoluble, so that they in turn do not diffuse away from the site where they are formed. The substrate must, of course, be acted on by the enzyme, but it need not necessarily be (and usually is not) identical with the substrate which is acted on *in vivo*.

The technique for the demonstration of alkaline phosphatase is a good example of this kind of histochemical procedure. This enzyme occurs in many cells and tissues. In life, it acts on phosphate esters, hydrolizing them and splitting off the phosphate groups

$$\mathrm{R\text{–}H_2PO_4 + H_2O \xrightarrow{\text{phosphatase}} R\text{–}OH + H_3PO_4}$$

Histochemically this enzyme can be demonstrated by incubating sections of tissue in a solution containing a phosphate ester, such as sodium β-glycerophosphate. Such compounds are hydrolized by phosphatases, which are classed as acid or alkaline according to the pH at which they are most active. Hydrolysis results in the release of free phosphate ions into the solution. In order to obtain a precipitate at the site of enzymatic activity, the reaction for alkaline phosphatase is carried out in an alkaline solution containing an excess of calcium ions, which react with the phosphate ions as soon as they are liberated to form calcium phos-

phate. This is highly insoluble, and is precipitated at sites of the enzymatic activity.

$$Ca^{++} + HPO_4'' \rightarrow CaHPO_4$$

Crystals of calcium phosphate can be detected microscopically but they are somewhat difficult to see, particularly when present in small amounts. After incubation therefore the sections are treated with a solution of cobalt nitrate, which converts the calcium phosphate into a cobalt salt; treatment with a solution containing hydrogen sulphide then produces a black precipitate of cobalt sulphide, which is readily visible, and whose distribution should correspond to that of alkaline phosphatase in the fresh tissue.

Phosphatases may also be demonstrated by using as substrate calcium β-naphthyl phosphate, in the presence of a diazonium salt. The hydrolysis liberates β-naphthol, which combines with the diazonium compound to give a coloured insoluble dye, which reveals the sites of phosphatase activity (Plate III, Fig. 9).

FLUORESCENT ANTIBODY TECHNIQUE

Certain types of protein can be demonstrated in cells by using a technique which combines immunological and fluorescent methods. Essentially, an antibody to the type of protein to be studied is produced by injecting it into a living animal. The antibody is then isolated from the animal's plasma and conjugated with a fluorescent substance, so that the antibody is "labelled", and can be detected by illuminating it with ultra-violet light.

Suppose that the protein used to produce antibody was growth hormone of the anterior pituitary of rats; this, injected into rabbits, acts as an antigen, and the rabbits produce an "anti-rat-growth hormone substance", which is then rendered fluorescent. If now a section of rat's pituitary gland is treated with a solution containing this antibody, an antigen-antibody reaction will occur in cells containing growth hormone. Because of the nature of this reaction, antibody becomes localized in these cells, and since the antibody is coupled with fluorescent

material, its distribution can be determined by fluorescence microscopy, and hence those cells producing growth hormone identified. Unfortunately, the validity of this technique must depend on the purity of the initial immunizing protein, and this is often likely to be contaminated with other antigenic material; results must therefore be cautiously interpreted.

PREPARATION OF TISSUES FOR HISTOCHEMICAL TECHNIQUES

Some histochemical techniques can be carried out using specimens prepared by methods commonly used in histology; thus wax-embedded sections of material fixed in one of a variety of fixatives can be stained satisfactorily with methyl-green-pyronin. Many active substances, including most of the enzymes are, however, largely destroyed by many fixatives, or by the dehydration and embedding in molten paraffin, and sections prepared by such methods may retain little if any of the activity present in fresh tissue.

The damaging effects of dehydration and embedding can be largely avoided by the use of either a freezing microtome or a cryostat. These are devices for cutting sections from tissue which has been hardened by freezing instead of by infiltration with wax. The freezing microtome is the simplest; it consists of a hollow metal chuck on which the specimen is placed, which is cooled by allowing carbon dioxide, fed to it under pressure, to expand inside it. This lowers the temperature of the chuck, and freezes the specimen. Sections are cut from the frozen specimen by sliding a knife, held in a rigid clamp, across the block. Each stroke automatically raises the chuck by a predetermined amount, and sections of this thickness are cut from the block. The more complex cryostat, designed for the same purpose, is essentially a microtome enclosed in a refrigerated cabinet. It has the advantage over the freezing microtome that sections remain frozen while they are within the cabinet, and can be easily manipulated while still frozen, whereas with the freezing microtome they thaw as soon as they are cut.

Frozen sections can be cut from fresh unfixed material, or the specimen may be first fixed in some aqueous solution which does not destroy the enzymes, or extract other substances whose distribution is to be studied. An aqueous solution of formalin in saline is often used. Fixation often does in fact destroy some enzymatic activity, but it is more difficult to cut good sections from unfixed material, and diffusion artifacts are more likely to occur.

Freeze-drying is another technique applied to the preparation of tissues for histochemical studies. It is designed to immobilize the constituents of cells and tissues *in situ* by rapidly freezing them, and then to remove the water while the tissues are still frozen without exposing them to any dehydrating agents. The first step in the procedure is to cool small pieces of tissue as soon as they have been removed from the animal to a temperature approaching that of liquid air, i.e. about −180°C. Cooling must be as rapid as possible to minimize diffusion artifacts and to prevent the formation of ice crystals which would distort the tissue. It is not enough simply to plunge pieces of tissue into a bath of liquid nitrogen, since the heat of the tissue vaporizes the gas, and it forms an insulating layer which slows down the cooling process. Instead, the tissue is cooled, or "quenched", in a bath of propane and isopentane cooled by the liquid gas; this remains in close contact with the tissue, and provided the specimen measures less than a millimetre or two in thickness, rapid cooling is effected.

After quenching, the tissue is rapidly transferred to a tube immersed in a mixture of solid carbon dioxide and acetone, and while still frozen, exposed to a high vacuum. The water in the specimen, which is of course still present as ice, slowly sublimes, and is absorbed by a drying agent such as phosphorous pentoxide which is included in the system. By this means, over the course of a few days, the specimen is dehydrated without being exposed to any of the solvents usually used.

Following drying, the tissue is allowed to warm to room temperature, and then infiltrated with paraffin wax, so that sections can be cut on a rotary microtome. These sections are

still unfixed; they can either be treated with histochemical reagents in this state, or first exposed to some fixative such as formaldehyde vapour; they should not of course be treated with aqueous solutions, since this will immediately rehydrate the sections and allow diffusion of materials from their original position. The technique of freeze-drying tends to have limited applications, chiefly for studies of cells rather than tissues, since it is difficult to obtain large sections showing uniform preservation throughout.

AUTORADIOGRAPHY

The ready availability of radioactive isotopes in recent years has been accompanied by the increasing use of these materials in biological research. Isotopes are elements which, because of an increased number of neutrons in the atomic nucleus, have different atomic weights from those elements which they resemble chemically. The atomic nucleus of radioactive isotopes is unstable, and tends to break down forming a more stable element, and emitting in the process α or β particles, or γ rays. The most important characteristic of these elements from the biologist's point of view is that cells and tissues cannot readily distinguish compounds incorporating a radioactive element from naturally occurring compounds having the same chemical formula. Amino acids which have been labelled with radioactive isotopes of hydrogen or sulphur, for example, are utilized by cells over normal metabolic pathways, and are incorporated into structural components of tissues or into secretory products of cells, where they can be detected.

One method of determining the distribution of radioactive material in tissues is by autoradiography, which depends on the reduction of silver salts in a photographic emulsion by the emitted radiations. In practice the specimen of tissue containing active material is fixed and sectioned, often after infiltrating with paraffin wax in the usual way. The sections are then placed in close contact with a photographic emulsion, and stored in the dark for a period which depends on the strength of the emission

from the active element. The emulsion is then developed in the same way as any other photographic film, and this reveals the distribution of the reduced silver grains. The original section remains in contact with the emulsion, and can be stained; examination under the microscope will then show the relationship of the reduced silver grains to morphological features of the section. The sites of highest concentration of the labelled material will be associated with the greatest density of reduced silver grains and some quantitative assessment of the activity in various parts of the section can be made.

Autoradiography has provided an understanding of the dynamic aspects of cellular activity in a way which was not previously possible. Two illustrations of this will be given. The first of these is the "tagging" of cells by means of labelled thymidine. This is a nucleotide base, which is incorporated into the DNA of cell nuclei during the period when active synthesis of DNA is going on, that is during a limited part of the mitotic cycle. Thymidine can be labelled with tritium, which is a radioactive isotope of hydrogen. Labelled thymidine injected into an animal will become incorporated into the nuclei of cells which divide, and hence synthesize DNA, in the period immediately following the injection. These cells will then be labelled with radioactive material, and although the activity will be halved at each subsequent cell division, due to the halving of the original chromosomal content of DNA, such labelled cells can nevertheless be identified autoradiographically over a considerable period of time, depending on their rate of division.

This technique has been applied to the study of the lining epithelium of the intestine. Only cells at the bottom of the glandular crypts (Plate III, Fig. 10) take up the thymidine, and become isotopically labelled. Those over the tips of the villi are not mitotically active, and hence remain unlabelled. If samples of intestine are taken from a series of animals killed at varying times after injection of the active material, it is found that labelled cells ascend from the basal parts of the crypts towards the villi, eventually after about 36 hr reaching their tips and

being cast off into the lumen of the gut. Such a progression of cells from crypts to villi might be inferred from a study of mitotic activity in various parts of the mucosal lining of the intestine, but is demonstrated much more clearly by the technique described.

A second example of the value of autoradiography is provided by studies on the hypothalamus and pituitary gland. The posterior part of this gland is largely made up of the expanded processes of nerve cells lying in two groups on each side of the hypothalamus. Histological studies indicated that secretory material found in the posterior pituitary might be manufactured in these nerve cells, and pass down their processes to be stored in the pituitary. This secretory material is closely associated with two hormones, oxytocin and anti-diuretic hormone, which are both peptides, and contain a high proportion of the sulphur-containing amino acid cystine in their molecules.

Cystine can be isotopically labelled with radioactive sulphur, S_{35}. Injection of such labelled cystine into the ventricles of the brain is followed within a short interval by the appearance of radioactivity in the nerve cells of those groups connected with the posterior pituitary. Some hours later, activity can be detected in nerve fibres of the stalk connecting the hypothalamus to the gland; but only after a considerable period of time has elapsed does active material appear in the infundibular process itself. These observations suggest that cystine is first incorporated in secretory material within nerve cells of the hypothalamus, and that this substance, now under the experimental conditions labelled with radioactive cystine, is then passed down the processes of these nerve fibres, and eventually stored in their terminal expansions until such time as the active hormone is released into the bloodstream.

CORRELATION OF STRUCTURE AND FUNCTION

Quantitative studies

Biology is in some respects a less precise science than others such as physics or chemistry. The reason for this is twofold; in

the first place, the living organism is an extremely complex system whose activity, and hence the activity of its component organs, tissues and cells, is influenced by a great many factors. Both external, or environmental stimuli, and stimuli arising within the organism itself, modulate this activity, and both the relative importance and the functional significance of many of these stimuli is even now imperfectly appreciated. In the second place, most biological studies involve, perhaps to a greater degree than other disciplines, some element of interpretation by the observer, and however objective he may try to be, subjective bias is very difficult to exclude. Furthermore, it may be difficult to communicate observations with any great precision, particularly where descriptions of data such as the different staining characteristics of cells and tissues are involved. Photomicrographs may help in a problem such as this, but do not provide a complete solution, since there is a limit to the number of photographs which can be published, and coloured reproductions, besides being expensive, do not necessarily reproduce the true colours of the original slide.

Description of the microscopic structure of tissue has often been regarded as an end in itself, but studies of this kind often tend to be somewhat sterile, and what is needed is some attempt to correlate structure and function. Such a correlation can be made in many ways. The appearance of a gland producing digestive enzymes, for example, can be studied when the animal is fasting, and compared with the appearance when the animal has eaten; or the histological structure of the reproductive tract can be correlated with the various phases of the sexual cycle. The biologist himself may attempt to modify the activity of some organ or tissue by experimental means, and thereby hope to gain some understanding of its mode of activity. Experimental biology is largely based on studies of this kind; but attention must be paid to many details if the results obtained are to be of real value.

Consider as an example an experiment designed to determine the effect of removing the ovaries on the pituitary gland, as

part of an investigation of the functional relationship between the pituitary and the gonads. In such an experiment, it is necessary to compare the structure of pituitary glands of normal animals with those of animals whose ovaries have been removed. In order to reduce as much as possible the range of variation, it is preferable to use animals which resemble each other in as many respects as possible, and differ only in that the control animals still retain their ovaries, but that the ovaries have been removed from the experimental animals. Ideally, therefore, experimental and control animals should be pairs from the same litter, obviously of the same sex and age, and housed under identical conditions. Although the ovaries are left intact in the control animals, these should have been subjected to a "blank" operation, in which the ovaries were exposed but not otherwise interfered with. At the end of the experiment, pairs of animals should be killed at the same time, and the tissues for histological examination treated exactly alike. Study of the pituitary glands, in this example, should be made without the observer knowing which glands are from which animals, so that subjective bias does not influence his observations.

Qualitative studies can often tell us a great deal about functional aspects of structure; but it is often desirable when possible to make objective observations, and to collect measurements which express in fairly precise terms the results of an experimental procedure, and which can be subjected to a statistical analysis which will indicate how significant these results are. Quantitative methods of study can be applied to the problem considered above. The removal of the ovaries is followed by changes in the appearance and in the number of certain types of cell in the adenohypophysis, as a result of the loss of ovarian hormones from the bloodstream following the operation. The type of change can be determined by examining stained sections of the glands and comparing those from experimental and control animals. The degree of change, which must be known before it is possible to compare the effect of the procedure on glands of different animals on a quantitative basis, necessitates the estimation of

the relative or absolute numbers of the different types of cell in the gland, again of course comparing the values obtained for experimental and control animals.

An estimation of this kind involves counting cells in sections taken from comparable parts of the different glands. It is essentially a sampling technique, since it is impossible to count all the cells in every gland; hence, since the cellular make-up of the gland often varies from one part to another, it is necessary to ensure that no area differing greatly in its cellular content from others is missed through inadequate sampling. Changes in size of the cells must also be allowed for, since if cells increase in size, the actual volume of such cells in the gland may be increased without any increase in their number. There is no need to go into the practical aspects of these problems here; it is enough to note the problems, and to say that due allowance can be made for the possible errors in estimations.

In other kinds of experiment it may be necessary to determine the total number of cells in a certain group. We might want to know, for example, how many cells remain in a nucleus of nerve-cells in the central nervous system after a nerve which originates from this nucleus has been cut. In such a case, an estimate of the total number of cells can be made by counting all those in sections taken at intervals throughout the nucleus, and comparing the figures obtained with those for a similar group of cells which have not been affected by the experimental procedure, such as those on the opposite side of the brain. Here again certain precautions must be taken to avoid errors caused by variations in closeness of packing of the cells, or by any particular orientation of cells in the group.

The size of cells may in itself indicate the level of their activity. The epithelium lining the follicles of the thyroid gland, for example, is low when the gland is inactive, but increases in height when the gland is stimulated by the thyrotrophic hormone from the adenohypophysis. Measurements of the height of follicular cells chosen at random from various parts of the experimental gland, and compared with similar measurements from a control

gland, can be used as an index of the effect of experimental procedures, such as the administration of drugs or hormones, on the output of thyrotrophic hormone by the pituitary.

Histochemical characteristics may also give some indication of the intensity of cellular activity. Cytoplasmic basophilia due to the presence of RNA, for example (see p. 15), is often associated with active synthesis of protein; and certain enzymes, such as phosphatase, may also be associated with synthetic mechanisms. The cellular content of RNA or of enzymes varies in different phases of cellular activity, and this variation may be revealed by changes in the intensity of the histochemical reaction used to demonstrate the substance in question. But while biochemical estimations of the amount of an enzyme or other substance present can be made with considerable accuracy, quantitative histochemical estimations are more difficult to achieve. The intensity of a histochemical reaction can be assessed as negative, or of moderate or high intensity with relative ease; but it is much more difficult to determine intermediate points on such a scale.

Some of the problems involved in quantitative histochemistry have been studied by Caspersson and his colleagues working at the Karolynska Institute in Stockholm over the last twenty years or so. This group of workers has pioneered the technique known as quantitative microspectrophotometry. In principle the technique is relatively simple, but in practice difficult. It depends essentially on measuring the amount of light transmitted (or absorbed) by a substance for different wavelengths of incident illumination. In practice light of wavelengths lying in the visible and ultraviolet parts of the spectrum is usually used, and the figures obtained are plotted as an "absorption curve". The value of the technique largely depends on the fact that this absorption curve is often typical for a given substance, such as nucleoprotein, so that from a study of the curve obtained the amount of this substance present in the specimen can be deduced.

A major problem in the application of this technique to individual cells is that these are very small structures, and also that the amount of, for example, nucleic acid present varies

considerably along any line passing across the cell. The amount of such a substance may be high in some parts of the cytoplasm, low in others, high in the nucleolus, and low in other regions of the nucleus. Nevertheless, despite practical difficulties, quantitative data for the distribution of nucleoprotein in many types of cell have already been obtained, during stages of development, and during cycles of secretory or synthetic activity.

EXAMINATION OF LIVING TISSUES

Tissue culture

The phase contrast microscope has already been briefly referred to on p. 6. The introduction of this instrument as a tool in biological research has made possible the examination of living tissues, or of unfixed and unstained specimens, by enabling cellular detail to be distinguished which cannot be seen in such preparations with the ordinary optical microscope.

Direct examination of living tissues while these are still within the animal is necessarily limited (see below) and it is usually impossible to study, at high magnification, cells of most tissues in their natural environment. Small pieces of tissue can however survive and grow in artificial media, and this is the basis of the technique of tissue culture. Provided that the container in which such tissues are grown is optically suitable, and that the specimen is not too dense to allow light to be transmitted through it, tissues in culture can be examined microscopically while they are fully active, although they are of course in an abnormal environment.

A few attempts to explant tissue from animals were made around the beginning of the present century; but it was Harrison in 1907 who showed that cells could continue their normal activity in an artificial medium. He transplanted pieces taken from the developing nervous system of frog embryos, and found that the nerve-cells continued to grow and differentiate, and axonal processes developed, when such fragments were cultivated in a clot of lymph.

The simplest method of cultivation of tissue outside the organism is that which uses the hanging-drop technique. In this, cells are grown in a drop of suitable medium on the under surface of a coverslip, which is placed over a depression in a glass microscope slide (Fig. 2). Many more complex arrangements have been

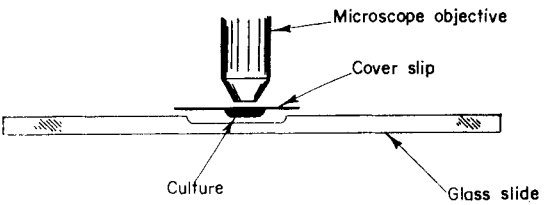


FIG. 2. Diagram of the hanging-drop method of tissue culture.

devised to meet the requirements for long-term culture. In such cases, the medium must be kept sterile and free from contaminating bacteria; the pH and osmotic pressure must be carefully controlled; the supply of essential amino acids, vitamins, sugars, inorganic ions and gases must be maintained, and waste products must be removed. By successfully transferring cells from a culture to a new batch of the medium, or "explanting", it has been possible to maintain strains of some cells living and growing for many years.

Transparent chambers

Early attempts to study living tissues were made during the last century before the introduction of tissue culture. Ziegler in 1875 reported a method which involved the implanting of a pair of coverslips, separated by a narrow gap, into subcutaneous tissue or muscle of a living animal. The space between the coverslips filled with tissue fluid and, connective tissue cells grew in. The coverslips could then be removed from the animal, and examined immediately afterwards, while the cells were still living. The limitation of this method was that after removal from the animal, the cells between the coverslips did not live for much more than an hour, which left little time for observation.

A technique making possible the prolonged study of living tissues in the animal was introduced by Sandison in 1924. Sandison implanted a small transparent chamber, made of two layers of mica separated by a space, in the ear of a rabbit (Fig. 3).

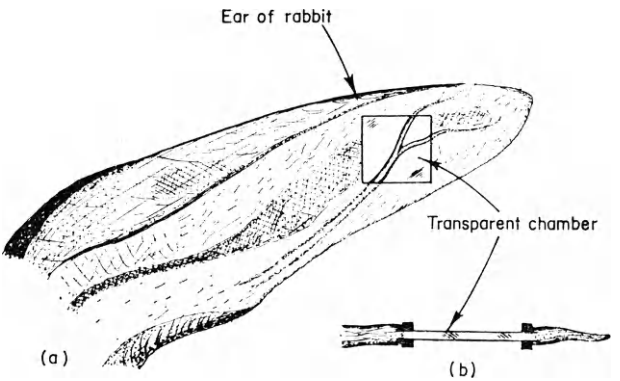


FIG. 3. (a) Transparent chamber in the ear of a rabbit, (b) shows the appearance in section.

The chamber was sutured into place, and became filled with tissue fluid; in some cases the chamber was so arranged that a piece of tissue of the ear, such as cartilage, projected into it. In either event, various elements of connective tissue grew in between the mica windows, including fibroblasts and various wandering cells. Intercellular material was laid down, and the whole process was accompanied by the growth of blood and lymphatic vessels. The contents of such a chamber could be easily examined with the high powers of the microscope while the animal was still living, and thus it was possible by intermittent observations to follow the progressive growth of the various components of connective tissue. This technique has also been applied to the study of grafts of tissue, and in such cases also the growth and vascularization of a graft can be observed with the microscope.

Valuable work has been done using the natural "transparent

chamber" that is formed by the anterior chamber of the eye. Small pieces of tissue can easily be inserted into this through a small corneal incision. Successful grafts become attached to the iris, and receive a blood-supply from its vascular bed. The vascularization and growth of such a graft can be easily followed, since the tissue is usually clearly visible through the transparent cornea. This technique does not, however, lend itself to high power examination of the transplant.

Quartz rod technique

It is quite easy to observe structures lying superficially in tissues of the living animal using a high-powered binocular microscope with a powerful source of illumination. For example, the pituitary stalk can be exposed with the animal anaesthetized, and the superficial vessels in the stalk can be examined, using incident illumination. Any study which requires the use of transmitted light, however, comes up against the problem of how to conduct light of sufficient intensity to the tissue without damaging it, since the directing of a powerful light source on to tissue is likely to lead to overheating. Attempts were made to overcome this problem by using rods of glass or other material to conduct light by means of internal reflection from a suitable source to the tissues. A considerable technical advance was made by Knisely, who in 1936 published details of a method using fused quartz rods to conduct the light. Quartz has the advantage over glass and other materials that less light is lost during transmission along the rod, and hence the illumination of the specimen is better. At the time when Knisely introduced this technique, a lively debate centred on the pattern of the circulation through the spleen. It was not known whether this was "open" or "closed", in other words, whether or not there were actual discontinuities in the walls of the intrasplenic sinusoids which allowed red blood cells to pass directly into the splenic pulp. The quartz rod technique proved very suitable for this type of study, since it enables the splenic circulation in the spleens of small animals to

be studied microscopically by transmitted light while the animal was still living. The technique can also readily be applied to the study of tissues such as the mesentery or intestinal wall, and it provides another technique by means of which some tissues can be examined while still part of the living animal.

CHAPTER II

Components of tissues

CELLS

All tissues consist of cells and intercellular materials. The properties of any tissue depend on the relative proportions of these two components and on their general arrangement, as well as on their specific characteristics. Functional specialization of any tissue is reflected in its overall structural organization, as well as in the detailed morphology of individual components.

Cells are essential living elements of tissues, and they vary greatly both in structure and in function. Some cells are relatively unspecialized or undifferentiated, while others have developed highly characteristic morphological and functional attributes, which make them readily recognizable. There is, however, a basic pattern of structure common to all cells, and this will be described before specialized cells are dealt with.

Details of cellular structure which can be appreciated with the optical microscope are necessarily limited by the degree of resolution obtainable with this instrument, and our knowledge of the fine structure of cells has been greatly advanced by the electron microscope (Fig. 4). Light microscopy does not permit the study of the internal organization of various structures found in cells, such as mitochondria and the Golgi apparatus, although it will show the presence of these organelles within cytoplasm. Nevertheless, despite its limitations, light microscopy can provide a great deal of information about the morphology of cells and their arrangement in tissues; and, together with specialized techniques such as histochemistry, can enable us to build up a dynamic

picture of the activity going on in the various tissues of the animal.

A typical cell consists of a nucleus and surrounding cytoplasm. The nucleus is usually round or oval, and may be indented. It is separated from the cytoplasm by a nuclear membrane, although

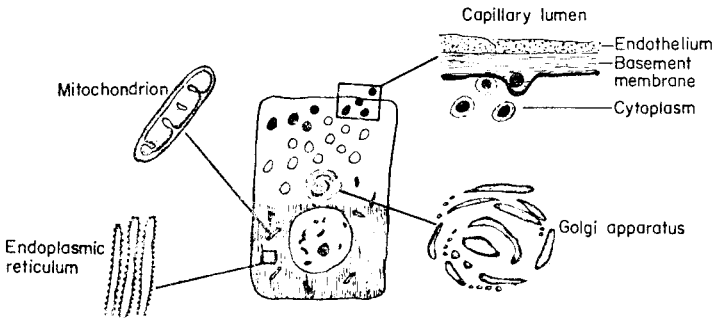


FIG. 4. Diagram to show the main components of a secretory cell visible with the optical microscope (centre), and the greater detail which can be shown with the electron microscope. The upper right-hand diagram shows the passage of secretory granules out of the cell towards a capillary.

the actual membrane itself is invisible under the light microscope. The resting nucleus, in the interval between successive cell divisions (interphase), contains unstained nuclear sap in which lie scattered granules of chromatin, and a rounded body, the nucleolus. The chromatin and nucleolus are basophilic and stain with dyes such as haematoxylin or toluidine blue. Histochemically it can be shown that chromatin contains a high proportion of DNA, while the nucleolus consists largely of RNA, but has a small amount of "nucleolar-applied chromatin" containing DNA.

DNA is an essential component of the chromosomes, or more specifically of the genes, which are the discrete units responsible for the transmission of inherited characteristics from one generation of cells to another, and via the germ cells, from one generation of individuals to the next. In the interphase nucleus, however,

the chromosomes cannot be distinguished as formed structures, and the chromatin appears dispersed throughout the nucleus (see p. 57).

Intranuclear RNA is concentrated in the nucleolus. This body plays a major role in the synthesis of protein, and is believed to be directly responsible for synthesis of RNA. During active synthesis the nucleolus enlarges, and a prominent nucleolus is a characteristic of rapidly dividing cells, such as those in many foetal tissues. In such cells there are also masses of material rich in RNA around the nuclear membrane, and usually there is a high content of RNA in the cytoplasm. Studies using precursors of both RNA and other proteins, labelled with radioactive isotopes, have clearly shown that the nucleolus is involved in a synthetic process, and these labelled precursors are incorporated into the nucleoli in far greater amounts than any other constituents of the cell.

A histochemical method for the demonstration of DNA and RNA has already been described (see p. 15), and this can be applied to the demonstration of these nucleic acids in nuclei. Other histochemical techniques, such as those for phosphatases, often show a positive reaction in nuclei, but there has been considerable debate as to whether this really indicates the presence of these enzymes within the nuclear envelope, or whether the result is an artifact due either to adsorption of the enzymes from the incubating solution on to intranuclear structures, or to diffusion of the reaction product from nearby positive sites. A number of enzymes has however been demonstrated in nuclei, but most of our knowledge of the chemistry of these elements has come from studies applying biochemical methods to preparations of isolated nuclei, rather than from histochemical work.

The cytoplasm constitutes the rest of the cell, and surrounds the nucleus. It also is bounded externally by a cytoplasmic or cell membrane, whose structure, like that of the nuclear membrane, can best be studied with the electron microscope. The cytoplasm itself consists of a homogeneous translucent ground

substance, in which lie various specialized bodies, the cytoplasmic organelles. The ground cytoplasm in fixed tissue often appears finely granular or meshlike in appearance, although this varies both with the type of cell and with the method of fixation employed.

All cells contain mitochondria. These are structures of variable size and shape, and often appear as small granules or rods. They are easily destroyed by certain fixatives, but when preserved they may be mistaken for secretory granules, or even bacteria. They are preserved by oxidizing agents such as potassium dichromate, and after fixation in a suitable mixture can be readily stained by iron haematoxylin. They can also be stained in unfixed or living cells by immersing the tissue in a weak solution of the dye Janus green. This method of staining is called "supravital", and the mitochondria alone take up the stain.

The light microscope fails to reveal any internal structure in mitochondria, although they may sometimes appear to be swollen, or vesicular; electron microscopy, however, shows that they have a characteristic internal structure. Mitochondria are sites of metabolic activity, and both histochemical studies, as well as biochemical analyses of isolated mitochondria, have shown that these organelles are closely associated with oxidative enzymes such as succinic dehydrogenase and cytochrome oxidase.

The Golgi apparatus is another cytoplasmic structure present in almost all types of vertebrate cells. It was first described in nerve cells by Golgi, and since this original report a complex and confusing literature has grown up around this body. For many years the true nature of the apparatus was debated, and some even doubted its existence, dismissing it as an artifact produced during the preparation of the tissue for examination. Electron microscopic studies, however, have shown conclusively that it is a real component of the cytoplasm.

The Golgi apparatus can be studied to a limited extent with the light microscope in tissues treated with metallic salts, or with osmium tetroxide. The form of the apparatus varies from one type of cell to another, and also in cells of the same type at

different stages of activity. The commonest form is a reticulum of anastomosing trabeculae, but it may appear as rings or spheres. It may surround the nucleus, as it does in nerve cells, or lie to one side. In exocrine glandular cells it usually lies between the nucleus and the excretory pole of the cell. The apparatus can often be seen in cells which have not been treated to demonstrate specifically the Golgi apparatus as an unstained area of the cytoplasm. Details of the fine structure of the Golgi complex are shown by electron micrographs, in which it can be seen to consist of systems of membranous lamellae associated with small vesicles.

Functionally the apparatus appears to be closely associated with the formation of secretory granules, and these can sometimes be demonstrated lying within it. In some actively synthetic cells, such as those of the pituitary gland, acid phosphatase can be demonstrated in high concentration in the Golgi zone, but not elsewhere in the cytoplasm.

Other cytoplasmic inclusions occurring in many different types of cells can be demonstrated by electron microscopy, but are too small for study with the optical microscope, since they are below its limit of resolution. Such components may, however, modify the staining reactions of the cytoplasm; thus cells which are actively synthesizing protein contain large amounts of cytoplasmic RNA, which is readily detectable by its affinity for pyronin. Studies with the electron microscope have shown that RNA, associated with protein as ribonucleoprotein (RNP), may occur as small granules lying free in the cytoplasm, known as ribosomes; or may be associated with a complex system of membranes called the endoplasmic reticulum. This basophilic material, rich in RNA, has often been referred to as the ergastoplasm. It is particularly prominent, both at the light and electron microscope levels, in cells such as those of the exocrine pancreas, which are very active in the synthesis of protein.

Glandular cells usually contain secretory granules, and often these are above the limits of resolution of the light microscope. Frequently, however, the coarse granules seen in the cytoplasm

have been formed by the aggregation of much smaller granules during fixation. Variations in the amount of granules in secretory cells may indicate whether the cells are in a phase of active synthesis, storage or excretion, but care must be taken when any attempt is made to infer stages of activity from histological sections, since except under special circumstances, a cell can only be examined microscopically at one point of its cycle of activity. In any event an abundance of secretory granules in a cell does not necessarily indicate a high level of synthetic activity at the time of fixation, but may be associated with a phase of storage. Active synthesis on the other hand may be associated with few cytoplasmic granules, although there may be other indications of activity, such as a high cytoplasmic content of RNA or phosphatase, or nucleolar enlargement.

CELLULAR SPECIALIZATIONS

A large proportion of cells in the body are specialized to play some particular role in the functioning of the whole organism. This specialization is reflected in the morphology of the cells, and some indication of the type of activity going on in cells can be gained from their histological and histochemical characteristics.

Many cells play more than one role; but it is perhaps useful to consider in the first instance the characteristics of cells in relation to their primary function. On this basis, cells can be broadly divided into classes: "active" and "passive" protective cells; absorptive; secretory; conducting; and supporting elements. As a preliminary to considering the complexities of the various types of tissue of the body, it may be useful to discuss in a general way the specialized features of cells of these various categories.

PROTECTIVE CELLS

A distinction can be made between active and passive protective cells. Active protection involves such actions as the ingestion of foreign particles and bacteria, or the elaboration of agents which act against foreign protein introduced into the body. Passive protection may take the form of mechanical resistance

to penetration by harmful agents, or protection of deeper-lying tissues from mechanical damage.

Cells which are actively protective generally lack any precise and fixed relationship to each other, and in this respect can be contrasted with, for example, ephithelial cells, which are closely bound together to form a distinctive kind of cellular tissue. Active protective cells are often mobile, at any rate at some stage, and are either carried passively in the blood or lymph, or are capable of independent movement. Thus cells which are capable of ingesting foreign material or organisms such as bacteria move actively or passively to the part of the body where they are required, for example to an infected wound. Cells with this property of ingestion are called macrophages; they may be free, lying in connective tissue, or fixed, when they are attached to a framework of reticular fibres. Fixed macrophages occur in the lining of certain blood vessels or lymph spaces, such as those of the liver and lymph nodes. Such fixed cells, however, retain the potentiality of becoming free and migrating to other sites.

Cells of this category tend to have no characteristic shape; if lying free in connective tissues they are often rounded, while those lining vessels may be somewhat flattened, but not to the extent of true endothelial cells. Light microscopy reveals no striking morphological features, and the actual process of ingestion of foreign material can of course only be observed when the cells are living, as in tissue culture. Cells of this type can however be recognized in fixed and stained material by the presence of ingested material in their cytoplasm, or at any rate by the presence of residual debris left after the break-down of whatever was ingested, Macrophages of the spleen, for example, often contain brown granules of the iron-rich haem pigment from broken-down red blood cells.

The presence of foreign protein in the tissues of an animal evokes a particular kind of protective reaction. The protein may be of bacterial origin, or from tissues grafted from another individual. It is called an antigen, and stimulates certain reactive cells in the host to produce a substance called an antibody, which reacts

with the antigen and inactivates it. The reaction appears to be a function of a class of cells called plasma cells, which are found particularly in lymph nodes and the spleen, and which appear in large numbers after the injection of a foreign protein into the animal. The cells are usually round or oval, with a darkly staining nucleus; often there is a relatively clear area in the cytoplasm to one side of the nucleus. Characteristically such cells have a high concentration of RNA in their cytoplasm, which can be demonstrated in the usual way by staining with pyronin.

PASSIVE PROTECTIVE CELLS

Many cells are specialized to fulfil a protective function in a passive way. They may simply line hollow organs such as blood-vessels; in this case the flattened endothelial cells have little cytoplasm, and offer minimal resistance to the flow of blood. Similar flattened cells, called now mesothelial, line the abdominal and thoracic cavities. In these sites the cells lie on a base of vascular connective tissue and form the pleural and peritoneal membranes, whose surfaces, moistened with a small amount of fluid, present little frictional resistance to the movement of organs relative to each other, or to the walls of the cavities.

Other types of protective cell have not undergone such a marked diminution in the amount of their cytoplasm as endothelial cells, but have acquired other characteristics which enhance their protective function. The skin, and the junctional zones where the alimentary and respiratory systems open on to the surface of the body, are covered by cells arranged in a number of layers. The deeper cells are cuboidal in shape, but those nearer the surface become progressively more flattened or squamous; the whole forms a protective stratified squamous epithlium. The deepest cells are relatively undifferentiated, and retain the ability to divide and form new cells to replace the more superficial ones which are constantly being shed, or desquamated, partly at any rate on account of the mechanical stresses to which such epithelium is continually subjected.

Cells of the epidermis not only become flattened as they

approach the surface, but they also change physically and chemically. Water is lost, and the fibrous protein, keratin, is laid down in their cytoplasm. This process of keratinization leads to a toughening of the epithelium. Keratin has a high content of amino acids, and especially of cystine, which contains a high proportion of sulphur in its molecule. During the process of keratinization sulphydryl (SH) groups present in the amino acids of the protein of the deepest layers of cells are oxidized to disulphide (SS) groups and, histochemically keratin can be distinguished by staining methods which reveal its high content of the latter groups.

A further feature of the epidermis is the presence within the cells of the brown pigment melanin, which has already been discussed on p. 12. Melanin is injected into the basal epidermal cells from the pigment-forming dendritic cells which lie along the junction of the epidermis and underlying connective tissue. The pigment remains in the epidermal cells as they migrate towards the surface; in the deeper and more cuboidal cells it is localized to form a cap over the superficial aspect of the nucleus, a position in which it acts as a shield to absorb harmful ultraviolet radiation, thus preventing damage to the nucleus. In the more flattened superficial cells, where the characteristic cytological features are lost, pigment granules can still be discerned, but they have no special disposition within the structureless keratinized plates into which the cells have developed.

SECRETORY CELLS

Some secretory cells also have a protective function; those lining the gut, for example, secrete mucus, which both reduces friction between the contents of the gut and its walls, and also forms a protective layer against the digestive enzymes. Nevertheless secretory cells of different types have many features in common, and despite any secondary functions such as protection, it is logical to consider secretory activity as a distinguishing functional characteristic which is reflected in structural organization.

Secretory cells are either exocrine or endocrine. The former type liberate their secretory products either on to some epithelial surface such as the lining of the gut, or into the epithelial-lined tubules of a system of ducts which conveys the secretion to its destination. Endocrine cells on the other hand release their secretions directly into the blood without making use of any system of ducts. Collections of cells of one or other type constitute exocrine or endocrine glands.

Both types of cell have certain features in common. They often show well defined stages of activity, in which they elaborate, store and release their products. These phases constitute the secretory cycle, although one phase is not necessarily sharply divided from another, and elaboration, storage and liberation can all occur to some extent at the same time. The cells may also pass through a resting phase, during which they are neither synthesizing nor storing material.

As already described on page 37 the most characteristic feature of secretory cells is the presence of secretory material, often in the form of granules, in their cytoplasm. These may be small, and even below the limits of resolution of the light microscope, but often they become clumped together during fixation and form larger readily visible granules. Some secretory material may not be preserved by certain fixatives.

Many secretory products take up stains used routinely in histology; granules in acidophil cells of the anterior pituitary for example, stain with acid dyes such as eosin or orange G, while exocrine cells of the pancreas take up a wide variety of basic dyes. Some secretory material contains particular chemical groups which can be demonstrated by specific histochemical reactions. Certain cells of the anterior pituitary, long known as "basophils", contain a mucoprotein which can be stained by the PAS technique. Since the stainable material in this case is closely associated, if not identical, with the hormones produced by these cells, such a histochemical stain offers a means of identifying the biologically active material *in situ*. Cells of the liver are similar in this respect, since they accumulate glycogen in their cytoplasm,

and this carbohydrate can also be readily stained by the PAS reaction. Other granules, such as those of mast cells (see p. 53) stain metachromatically, i.e. in a colour different from that of the dye used, a reaction which probably indicates the presence of polymerized mucopolysaccharides; and amino-acids such as tryptophan can be demonstrated in protein-containing granules, such as those of exocrine pancreatic cells.

Secretory cells are often polarized, that is they are often orientated in some specific way towards blood-vessels, ducts, or an epithelial surface. Thus the nuclei of mucus-secreting cells of the intestine lie basally, and mucus accumulates in the more superficial part of the cytoplasm, so that this part of the cell becomes distended; on account of their shape, these are called goblet cells. Exocrine secretory cells usually show polarization relative both to blood vessels, which lie basally, and on which the cells rely for the supply of metabolites used in the synthesis of their secretory products, and to the excretory ducts, along which the secretion must be passed. These usually lie on the opposite side of the cell from the vessels. Polarization in endocrine cells is often less striking, since they tend to be rounded or cuboidal rather than columnar, and since furthermore their secretions pass directly into the bloodstream, so that no system of ducts is present. Some endocrine cells, such as those of the thyroid gland which are arranged in follicles, do however show a marked polarization.

The Golgi apparatus of secretory cells is often conspicuous. It usually lies near the nucleus and, in polarized cells, usually on that side of the nucleus nearest the excretory pole. The apparatus is larger in cells in a phase of active synthetic activity. In hyperactive cells, which may be empty of secretory material because this is passed out of the cell as soon as it is formed, a few granules may be detected in the Golgi zone. In some secretory cells, a high concentration of acid phosphatase in the Golgi zone is associated with synthetic activity.

It has already been noted that some secretory material may be recognizable by a specific histochemical reaction; but in

addition to this, secretory cells may also show histochemical characteristics of a more general nature, related to the kind of synthetic activity which goes on in their cytoplasm. Cells of the exocrine pancreas, which synthesize proteins, contain large amounts of RNA, in marked contrast to the cells of the islets of Langerhans, forming the endocrine pancreas, which, although they are engaged in the synthesis of insulin, show only slight cytoplasmic basophilia (Plate II, Fig. 8).

It is customary to describe three distinct modes of secretion in glandular cells. These can usually be recognized by microscopy, provided that cells at various stages of the secretory cycle are examined. The first, eccrine secretion, is the commonest; this is the process in which secretory material leaves the cell by passing through its wall without disrupting it. Substances of small molecular weight can probably pass with minimal effects on the cell membrane; but larger granules, or substances of high molecular weight can also pass, as shown by large granules sometimes lying extracellularly near the cells which have elaborated them.

Studies with the electron microscope have clarified our ideas on this part of the secretory process. They have shown that secretory granules are usually bounded by a membrane. During the process of excretion from the cell, the granule moves towards the cell membrane, and the membrane bounding the granule fuses with that of the cell. The outer part of the membrane then breaks down at one point, so that the granule lies extracellularly, without there having been a complete rupture of the cell membrane.

The second mode of secretion is called apocrine. Here the secretory material elaborated by the cell accumulates in the distal part of the cytoplasm between the cell nucleus and the lumen of the duct. This material is then discharged by a shedding of the whole of the distal part of the cell; the basal part, containing the nucleus, remains, and the cycle begins again. This type of secretion occurs, in the human, in certain types of large sweat glands, such as those which are found in the axilla; and also in the mammary glands, which are in fact modified and specialized cutaneous glands.

The third type of secretory activity is called holocrine. Here the whole cytoplasm of the cell becomes filled with secretory material, and the cytoplasm becomes reduced to small septa between the secretory droplets. Release of the secretion occurs by disintegration of the cell, and so the material released consists both of secretory droplets and cytoplasmic debris. Sebaceous glands, which open into hair follicles, secrete in this way.

ABSORPTIVE CELLS

Cells whose main function is absorption may occur interspersed with other types of cell in an epithelial surface, such as the lining of the intestine, where both absorptive and secretory cells are found. Elsewhere, however, absorptive cells may be the only type present, as in the tubules of the kidney.

Absorptive cells may vary in shape from columnar to low cuboidal; the nucleus often lies basally, so that the cells are structurally as well as functionally polarized. The free border of the cell, which forms the actual absorptive surface, is often finely striated, and electron microscopy has shown that it consists of close-packed minute fingerlike projections from the cell membrane, called microvilli. If fixation has been satisfactory, small vacuoles can often be detected in the cytoplasm of absorptive cells; cells lining the intestine may contain small droplets of fat during the absorption of this material from the gut.

Histochemically, absorptive cells can be shown to contain enzymes believed to play a part in the absorptive process. Alkaline phosphatase occurs in the brush border of absorptive cells of the intestine and renal tubules, and may be involved in the splitting of phosphate esters to enable them to be transported across the cell membrane, and in the absorption of glucose. Phosphatase is also present in the endothelial lining of many blood vessels in tissues where active secretion is occurring, as in endocrine glands such as the pituitary and thyroid.

CONDUCTING OR NERVE CELLS

The activity of all organisms is controlled by stimuli arising

both from their external environment, and from their own tissues, that is their internal environment. Irritability, a reaction in response to a stimulus, is a fundamental property of protoplasm. This can be demonstrated in the unicellular amoeba. But in all except the simplest forms of living creatures specialized structures have developed for the reception of external and internal stimuli, their transformation into nervous impulses, transmission of these impulses and their correlation with others, and the conduction of excitatory effector stimuli to some tissue such as muscle through which the neural impulses are translated into activity.

In all but the most primitive creatures these activities are carried out by the nervous system, which reaches its greatest complexity in man. The fundamental unit in this system is the nerve cell or neurone. Neurones exhibit a tremendous range of variation in morphology, but all have certain basic features in common. As other cells, they have a nucleus surrounded by cytoplasm; but they differ from many cells in that the cytoplasm extends into processes which receive and transmit nervous stimuli (Fig. 5).

Most nerve cells have a number of branched processes, the dendrites, which receive impulses from other neurones; and a single process, the axon, which conducts impulses away from the cell body, and makes functional contact with other nerve cells, or with effector structures such as muscle fibres or secretory cells. The arrangement of neuronal processes, their number and length vary greatly. Some cells may have only two processes, and are called bipolar; others, pseudo-unipolar in type, give rise only to a single process which divides shortly after its origin; but most have a number of processes, and are classed as multipolar. The axons of some nerve cells, such as those lying in the spinal cord and innervating the distal muscles of the limbs, may be several feet in length; at the other extreme processes of some cells in the central nervous system which form part of a complex intercommunicating network of cells and fibres may all lie within a fraction of a millimetre of their origin.

Only a small proportion of neurones are secretory in the same way as glandular cells, but all are actively synthetic, and in common with other cells having this functional characteristic they usually contain a large amount of RNA in their cytoplasm. This

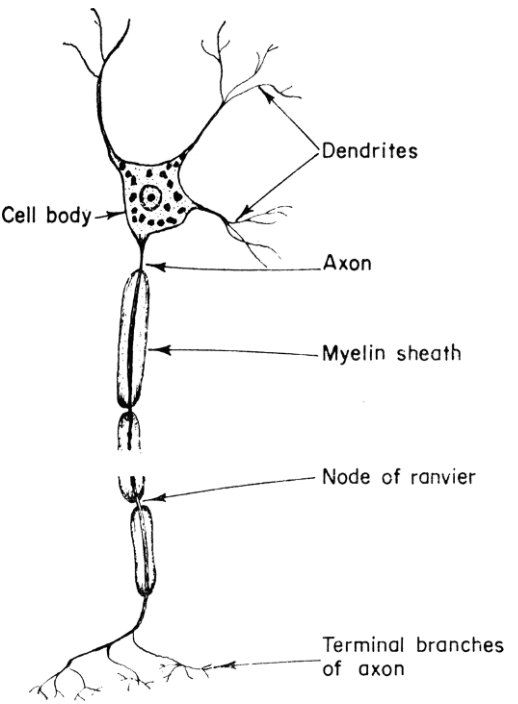


FIG. 5. Diagram of a nerve cell. The axon may reach several feet in length.

occurs in fine or coarse granules, called the Nissl material, which surrounds the nucleus and extend into the dendrites. Nissl material stains strongly with basic dyes; and in routine histological procedures it is commonly demonstrated by staining with aniline dyes such as toluidine blue or cresyl violet (Plate III, Figs. 11 and 12); dyes of this type are often called Nissl stains.

Another structural feature of nerve cells is the presence of fine fibrils, called neurofibrillae, in the cytoplasm of the cell body and processes. These are usually demonstrated by metallic impregnations. At one time there was much debate as to whether these fibrillae were in fact a structural component of neuronal cytoplasm, or whether they were artifacts produced during the preparation of the tissue for examination. They have now, however, been observed in living cells by means of the phase-contrast microscope, and are a constant feature of neurones when studied with the electron microscope. On such evidence it seems reasonable to assume that some fibrillary organization exists in the cytoplasm during life, although not necessarily in such a well-ordered form as that seen in fixed and stained material.

The axonal processes of all nerve cells of the peripheral nervous system are invested with a protoplasmic sheath called the neurilemma. This is derived from cells which grow out with the nerve fibres during development, each of these cells (called Schwann cells) investing a short length of the axon, so that the whole fibre is enclosed in a multicellular sheath. In the central nervous system, the place of the Schwann cells is taken by glial or satellite cells (see p. 110).

In addition to the neurilemma, large axons are also invested with a layer of fatty material called myelin. This is a lipo-protein complex which lies between the neurilemma and the surface of the axon. The myelin is interrupted at the junction between the territory of one Schwann cell and the next, at a point where the Schwann cell becomes closely applied to the axon; this region is called a node of Ranvier. The electron microscope has shown that myelin consists of tightly wound spirals derived from the cell membrane of the sheath cells. Fibres with a sheath of this kind, which is thick enough to be visible with the light microscope, are called myelinated, while fine fibres with no readily visible myelin sheath are classed as unmyelinated, although such fibres do in fact have a fine investing layer of Schwann cell cytoplasm.

Histological studies can provide evidence of the functional

state of neurones. Hyperactivity is accompanied by breakdown of the RNA-containing Nissl material, and after long-continued over-stimulation, for example, morphological evidence of neuronal damage becomes evident. Similar changes also follow damage to the cell or its processes. If an axon is cut the coarse granules of Nissl material in the cell body break down to finer ones, or may disappear altogether (Plate III, Figs. 11 and 12), and the nucleus of the cell moves from its central position to lie eccentrically. If damage to the neurone is sufficiently severe, degenerative changes may progress further and lead to death of the cell. Less severe damage may be followed by recovery; this commonly occurs during the regeneration of axons of a damaged peripheral nerve. Recovery of the neurone is accompanied by restoration of the Nissl material, and the return of the nucleus to its central position in the cell. The degenerative changes described above are known as chromatolysis.

Many nerve cells have a high concentration of acid phosphatase in their cytoplasm, and as already described this enzyme is often associated with synthetic activity. An increase in activity of neurones has been shown to be accompanied by an increase in the amount of this enzyme in the cells.

NEUROSECRETORY NEURONES

Neurones in different parts of the nervous system exhibit a wide range of variation in their morphology, which reflects their varied function in the conduction and correlation of nervous impulses. In one respect all neurones are secretory, since they elaborate chemical mediators which play an essential role in the transmission of a nervous impulse from one neurone to another, or to an effector cell (see p. 107). Certain nerve cells, however, show a different kind of specialization, and are responsible for the elaboration of active principles which may act at a distance from their site of release, and also act for a longer time than transmitter substances. Such principles are classed as neurosecretory hormones.

In mammals, nerve cells of the supraoptic and paraventricular

nuclei in the hypothalamus, which have already been referred to on p. 23, are neurosecretory. Processes of these neurones pass down the stalk of the pituitary gland, and their expanded terminal parts make up the infundibular process of that gland. The cell bodies of these neurones resemble those of non-neurosecretory nerve cells in many respects; thus they contain granules of Nissl material, although this tends to be distributed peripherally in the cell. They also contain a substance which can be specifically demonstrated by certain stains; this is the neurosecretory material, which appears to be closely related to the hormones elaborated by the cells.

CONTRACTILE CELLS

The simplest form of contractile cell in the mammal is the smooth muscle fibre. This is an elongated fusiform cell, which usually measures about 8–10 μ in diameter in its central region, but which may vary in length from 20–500 μ; the largest of these cells are found in the wall of the pregnant uterus. Each smooth muscle cell has a single nucleus, which lies about midway between the two ends of the cell, and as seen in transverse sections, may lie centrally or somewhat to one side of the fibre. In longitudinal sections the nuclei may appear wrinkled to a varying degree depending on the state of contraction of the fibre. The cytoplasm may appear homogenous, or may show a longitudinal fibrillar structure, due to the fact that the fibre is made up of small contractile units, the myofibrils, which are orientated along the length of the fibre, lying in a homogenous and more fluid medium, the sarcoplasm.

The cells which make up the greater bulk of the muscular tissue of the body have a more complex structure. In the first place they are much larger cells, measuring up to 40 μ in diameter, and sometimes up to 40 mm in length. Each cell or fibre contains many nuclei, which are distributed along its length, and tend to lie peripherally, as can easily be seen in transverse sections. The most distinctive histological feature of these cells is that they are cross-striated.

As in the case of the smooth muscle cells, striated muscle fibres are made up of small myofibrils lying in an optically homogeneous sarcoplasm. In transverse sections individual myofibrils can be seen, and these frequently form groups within fibres which appear as "islands" of myofibrils separated from neighbouring groups by a narrow zone of lightly stained sarcoplasm. The cross-striations are prominent in longitudinal sections of the fibres, or in teased preparations of whole fibres. They consist of alternate light and dark bands, each of which is divided across its central zone by a very narrow band with different staining properties. The banding across the whole fibre is itself dependent on the cross-striations of the myofibrils, which are aligned with each other so that the light and dark bands of adjacent units correspond, and the banding is consistent across the whole width of the fibre. The outer layer of each large fibre consists of an envelope, called the sarcolemma, which is derived from the fibre itself, and encloses the myofibrils, sarcoplasm and nuclei.

A third type of muscle cell occurs in the heart. This is also transversely striated, but differs from each of the two types already described in that individual elements are not separated structurally from their neighbours. Despite this continuity, however, it is clear that cardiac muscle is made up of individual units, each of which contains a single nucleus lying in the centre. This is often surrounded by a small area of sarcoplasm which is free from fibrils, but these fill the rest of the cell, arranged as in striated muscle fibres so that the banded pattern extends across the whole cell. At sites of continuity of one cell with those lying next to it there is a structure called an intercalated disc, which appears as a dark line. Side to side anastomoses also occur.

MYOEPITHELIAL CELLS

Myoepithelial cells are also contractile, but they are not strictly speaking muscle cells. They are found in relation to the epithelial cells of sweat, salivary and mammary glands. Typically they are cells which lie basally between the epithelium and the underlying connective tissue. Their cytoplasm extends into

branching processes which embrace the secretory cells. Myoepithelial elements are difficult to distinguish in ordinary histological preparations, but can be demonstrated by impregnating with silver salts. These cells contain a high concentration of alkaline phosphatase, and can be clearly demonstrated histochemically. Their function appears to be to express secretion from the acini along the ducts towards the surface openings of the larger ducts (see Plate III, Fig. 9).

CONNECTIVE TISSUE CELLS

Connective tissue contains a variety of cells which cannot easily be grouped under any one functional heading. Some of these, together with the intercellular material, are "supportive" in function; but protective, storage and other types of cell commonly occur.

As described later, connective tissues vary in structure according to the degree of specialization of their function, and such variation is also found in the type of cells present. Connective tissues play a mechanical role; one form constitutes the rigid bony skeleton; another, as tendons, serves to transmit the forces exerted by muscle on to the skeleton; dense connective tissue forms capsules for organs, and loose connective tissue invests bundles of nerves and vessels, extends between various tissues and organs, and also contains cells which are potentially protective and reparative, should the tissues be invaded by foreign organisms. Cells which serve as stores of food material, in the form of fat, are common in certain kinds of connective tissue. In all, intercellular material forms a major component, and the type and arrangement of this is largely responsible for the functional properties of the various kinds of connective tissue.

UNDIFFERENTIATED MESENCHYMAL CELLS AND FIBROBLASTS

These cells are found particularly in loose connective tissue. They are elements which have not become highly specialized, but have retained a potentiality for developing into fibroblasts

or other types of connective tissue cell. They are not readily distinguished from fibroblasts in stained sections, although the less differentiated cells tend to lie along blood vessels, while mature fibroblasts are more evenly distributed throughout the tissues. It should be pointed out here that mature connective tissue cells should really be called fibrocytes, since the suffix-blast denotes a primitive cell.

Fibroblasts are rather large irregularly shaped cells, whose cytoplasm extends into processes. In mature tissue, the cytoplasm stains rather poorly, but the oval nuclei of the cells can be seen, usually lying amongst dense collagen fibres. Both young and mature cells can be more easily studied in fresh material, such as a piece of loose connective tissue spread on a slide, rather than in sections. Methylene blue is a suitable stain for such a preparation.

Fibroblasts are concerned with the laying down of fibrous connective tissue, and can probably be regarded as a form of secretory cell. But before considering details of this mechanism, another type of cell which occurs in connective tissue, and which is also involved in formation of intercellular material, must be considered; this is the tissue mast cell.

Mast cells are heavily granulated elements which are widely distributed in loose connective tissue and in serous membranes, and are often disposed along blood vessels (Plate IV, Fig. 13). Similar cells are also found in the blood. They are large and oval, and their granules are basophilic, and also stain metachromatically. It was once thought that such cells were important only as the source of an anticoagulant, heparin; but more recently it has been demonstrated that they also contain histamine, a substance whose liberation from the cells is associated with inflammation. Histamine causes local dilatation of vessels and increased permeability; and substances which cause the release of histamine in tissues also cause swelling and disruption of mast cells.

THE FORMATION OF FIBROUS CONNECTIVE TISSUE

Fibrous connective tissue exists in two forms; reticulin and collagen. Reticulin consists of very fine fibres, which are best

demonstrated by metallic impregnations, which coat the fibres with a layer of submicroscopic particles of metal, or by the PAS reaction. Collagen consists of thicker fibres which stain readily with a number of dyes.

The way in which fibroblasts lay down fibrous tissue is not entirely understood, and cytologically these cells give little indication of their activity. Probably they polymerize organic materials to form a soluble "pre-collagen", which is secreted by the fibroblast into the surrounding medium. This soluble material is probably converted into reticulin by the addition of the sugar aldose and lipid material. Reticulin appears as delicate fibres in the intercellular matrix; it may then be converted into mature collagen by the addition of glucosamic acid, a process which is possibly accompanied by loss of other materials.

Since both reticulin and collagen contain carbohydrate, they both give positive reactions with the PAS reagent. It has indeed been suggested by Riley (1959) that the first stage in the formation of collagen is the elaboration of a carbohydrate material by fibroblasts, and that this material constitutes the structureless intercellular ground substance which is a precursor of formed connective tissue. Fibrils develop in the next stage, and Riley suggests that in this phase excess ground substance is broken down, and then reconstituted in a sulphated form as the anticoagulant heparin, which is stored in the granules of mast cells. Thus these cells may be involved together with fibroblasts in the formation of collagen, possibly acting as storage depots of mucopolysaccharides, which can be released at a later stage when they are required for the formation of more connective tissue. Heparin may occur in the blood simply as a result of the breakdown of mast cells, which liberate an excess of this mucopolysaccharide into the tissues. It must be pointed out that these interesting ideas are still somewhat speculative.

The formation of connective tissue from various simpler substances must involve the activity of a number of enzymes, but as yet there has been no clear histochemical demonstration of any characteristic chemical activity associated with the process.

It has been suggested that phosphatase plays a part in the formation of collagen, and this enzyme has been shown in large amounts in the tissues of healing wounds, where rapid new formation of collagen is occurring. An alternative explanation for this finding, however, is that the enzyme is merely adsorbed on to the newly formed collagen, and may not in fact be playing any active part in its formation.

FAT-CELLS

Fat, or lipid, may appear in cells as a result of injury, as a manifestation of a degenerative change; for example, after toxic damage to the liver by a substance such as carbon tetrachloride, fatty degeneration of the parenchymal cells occurs. Fat, however, is a normal component of connective tissue, and is stored within cells in large amounts. It first accumulates as small droplets in the cytoplasm, which as they increase in number coalesce, and eventually occupy the whole of the cell. The nucleus is displaced to one side, and the cytoplasm is reduced to a thin rim surrounding a large globule of fat. In some types of fat cell, the fat remains in separate droplets, which although closely packed together do not coalesce.

Wax-embedded material cannot be used to study fat, unless the specimen has previously been treated in some way so as to render the fat insoluble in the various solvents to which it is exposed during dehydration and clearing. Sections of wax-embedded material will therefore usually show only empty spaces from which the fat has dissolved; but such sections will often reveal quite clearly the distribution of fat, since the cells which previously contained it are quite characteristic; they are called "fat ghosts" (Plate IV, Fig. 14). The sites of small droplets, which had not produced any major distortion of the cytoplasm or displacement of the nucleus of the cell would however be missed. The simplest way of demonstrating fat is to fix the tissue in some aqueous fixative such as formalin, and cut sections on a freezing microtome, as described on p. 19. These can then be stained by dyes such as those of the Sudan group (see Plate IV, Figs. 15 and 16).

The dyes usually employed to demonstrate fat in sections do not act in the same way as most histological stains; in fact they are not true stains, but dyes which are particularly soluble in fat. Sections are exposed to a dilute solution of such a dye; fat globules preferentially dissolve it, and become coloured, while non-fatty components remain unstained. Stained sections must, of course, be mounted for examination in some medium which does not involve exposing them to fat solvents, since stained fat will be extracted as readily as unstained.

MISCELLANEOUS CELLS

Some of the other types of cell found in connective tissue have already been described. Free and fixed macrophages are among these. Plasma cells, and lymphocytes and other cells from the blood are common in some sites; while in some connective tissues, particularly that of the dermis of many animals, pigment cells occur and can be easily recognized by their content of light or dark brown pigment.

CELL DIVISION

Mitosis

Most nuclei in tissue from mature animals are in interphase, that is they are not involved in the active process of division at the time of fixation of the tissue. Dividing nuclei are found relatively frequently in tissues which are growing rapidly, such as those of the embryo, or those undergoing regeneration. The turnover of cells is also relatively high in some mature tissues, such as intestinal epithelium and skin, where cells are constantly being cast off from the surface; in other mature tissues, such as those of the nervous system, cell divisions are extremely rare; under normal conditions no new formation of neurones occurs after the developmental period.

The presence or absence of dividing cells in histological sections may indicate whether or not any appreciable new formation of cells is taking place; but a more reliable estimate can be made

by using some drug which arrests the process of division without preventing its onset. Such drugs are known as mitotic blocking agents. Colchicine is such a substance; if this drug is given to an animal in a suitable dose, mitosis is arrested before it is completed, but the onset of the division is not prevented. If an animal is killed say three hours after being given this drug, samples of tissue will show arrest in metaphase (see below) of all cells which have begun to divide during that time, provided of course that sufficient time has elapsed for the stage of metaphase to be reached. By counting the number of cells in metaphase, and comparing the figure obtained with the number of interphase nuclei present, an estimation of the rate of mitotic activity in a given tissue can be made.

As already described (p. 35) the interphase nucleus contains only scattered granules of chromatin and a round basophil body, the nucleolus; these inclusions lie in unstained nuclear sap, enclosed by the nuclear membrane. The granules of chromatin are associated with the chromosomes, which are the structures responsible for the transmission of inherited characteristics from one generation of cells to the next, and from one generation of individuals to another. Although the nuclear chromatin can be seen during interphase, the chromosomes themselves only appear as organized structures during the process of mitotic division (Plate V, Fig. 17).

Chromosomes consist of a pair of rod-like structures, the chromatids, which are joined together at one point, the centromere. The genetic material, which constitutes the genes, is distributed along the length of these rods. The total number of chromosomes varies with the species, but apart from pathological conditions, and polyploidy (a two or more fold increase found occasionally in some tissues) it is constant for each somatic cell of a given species.

The process of division of the cell falls into four stages, called prophase, metaphase, anaphase and telophase. The initial stage, prophase, is characterized by the appearance of the chromosomes as distinct bodies instead of scattered granules of chromatin, and

by the disappearance of the nucleolus and the nuclear membrane. The chromosomes at this stage are usually arranged in a complex coil. During the next stage, metaphase, the chromosomes become arranged to form a "plate" extending across the central part of the nucleus, and a conical spindle of fine fibrils extends from each side of the plate to a point situated just outside the position formerly occupied by the nuclear membrane. The spindle is probably an appearance due to the orientation of molecules along lines of force.

During the third stage of cell division, anaphase, the paired chromatids constituting each chromosome separate from each other, and appear to be drawn by the spindle fibres on each side towards its apex. The final phase, telophase, involves the formation of a new nuclear membrane around each set of chromosomes; a nucleolus is formed, and the cytoplasm becomes constricted between the two new nuclei, so that two new cells appear. In this phase the chromosomes disappear again as distinct bodies, and only scattered granules of chromatin of the interphase nuclei can be seen.

This process of mitotic division of the nucleus and cytoplasm to form two new cells results in the duplication of all the elements present in the original mother cell, so that each new nucleus possesses a full complement of chromosomes. Immediately after division, each new nucleus contains half the chromatids of the parent nucleus; but clearly the process of division must be associated with synthetic activity at some stage, otherwise with successive divisions there would be a progressive decrease in the amount of DNA and RNA in the newly formed nuclei. Probably division of the individual chromosomes to form two chromatids takes place late in the interphase before the beginning of the next mitosis, before the chromosomes are clearly visible. Thus synthetic activity would be associated with this stage.

Mitotic division is the common mechanism of formation of somatic cells. Another type of division, called meiotic, occurs in the reproductive glands, ovary and testis, during the formation of the germ cells. At one stage in the series of cell divisions which

give rise to mature ova in the female or spermatozoa in the male, a "reduction" division occurs, in which instead of all the chromosomes dividing so that each daughter cell has a full complement, one member of each pair of chromosomes separates to each pole, so that the two resulting nuclei have only half the normal complement found in somatic cells of the organism. This complement is only made up to the full number again after the ovum has been fertilized by the male germ cell. The halving of the original number of chromosomes, with subsequent restitution from a different source, is the mechanism whereby the genetic material of two separate individuals, contained in the chromosomes, is mingled to give a new individual incorporating characteristics of both mother and father.

CHAPTER III

The organization of tissues

Most tissues of animals are not made up of cells of a single type, but consist of a variety of cells and intercellular material. In addition, various formed structures such as blood vessels, lymphatics and nerves make up an integral part of tissues, and are essential for their function. It has already been seen that cells in different sites, which have some common activity such as secretion, also share many structural features, although they vary from each other in fine details. In the same way many tissues can be grouped together on a functional basis, and show morphological similarities which can be correlated with function. In this section, examples of some of the main types of tissues will be considered, in order to illustrate the relationship between structural organization and function.

EPITHELIAL TISSUE

Epithelial cells both cover surfaces, forming an epithelium, and also make up a large proportion of glandular tissues. Epithelium constitutes a rather special type of tissue, since it often consists of cells of a single type, and does not contain either blood vessels or lymphatics. In functional terms, however, it is wrong to consider epithelia in isolation, since they are always associated with connective tissue, and depend on this for their survival as well as for their function.

Epithelia are classified on a structural basis according to the type of cell they contain, and the arrangement of these cells relative to one another. These classifications are considered in

detail in textbooks of histology. From a functional point of view, two questions are of particular interest; firstly, how do the cells of an epithelium fit together and resist the mechanical stresses to which they are subjected; and secondly, what is the precise relationship of an epithelium to the underlying connective tissue?

In some sites, adjacent epithelial cells show a complex interlocking of their surfaces, but there is no good evidence that this plays any part in holding them together. A structural feature which is more clearly associated with attachment of cells to each other is the terminal bar. Such bars seem to consist of cement material lying between the most superficial parts of epithelial cells. Oblique sections passing through the surfaces of cells, and stained with iron haematoxylin, show the terminal bars as discrete black dots. Electron microscopy has revealed structures called desmosomes, which are probably identical with the terminal bars of light microscopy. At high magnification, each desmosome is seen to consist of a thickening of the cell membrane of adjacent cells, associated with an accumulation of electron-dense granular material, from which minute fibres radiate into the adjacent cytoplasm. The way in which these structures effect the adherence of cells to each other is not understood, since no fibrils or other structures actually pass from one cell to another.

The relationship of epithelium to underlying connective tissue is of fundamental importance. At the junctional zone between the cellular layer and the underlying connective tissue, the latter is usually modified to form a basement membrane. This consists of a thin layer of intercellular material which is not readily shown by ordinary staining methods, although it gives a strong positive reaction with the PAS reagent. This basement membrane is a specific structure, and not a condensation of reticular fibres, although these are present, apparently to reinforce the amorphous intercellular substance. Deep to the basement membrane there is usually a layer of connective tissue, which may be either dense, as the dermis of the skin, or loose, as in the submucosal layer of

the intestine. This underlying connective tissue contains blood vessels, nerves and lymphatics, as well as numbers of connective tissue cells. The survival of the epithelium depends on the diffusion of nutriments from vessels of the subepithelial connective tissue across the basement membrane.

Although epithelia are often examples of collections of cells of a single type, this is not always the case. In the first place, cells of a common origin may vary in structure in different parts of an epithelium; in the skin, to consider an example already discussed, the basal epidermal cells differ in appearance and in chemical and mechanical characteristics from those lying more superficially. Most of the different types of cell are essentially variants with a common origin; but other cells are also present which have a completely different origin. These are the pigmentary dendritic cells, also previously described, which lie in close relation to the basal epidermal cells, and via their dendritic processes inject pigment into the latter. These pigment-forming cells are derived from the neural crest, whereas the epidermal cells develop from somatic ectoderm.

The latter example cited two kinds of cell of diverse origin which had established a close functional as well as structural relationship to each other. In other instances the functional significance of an apparently alien type of cell lying amongst epithelial cells is not so clear. In the wall of the intestine, for example, accumulations of lymphocytes often lie in the subepithelial connective tissue; and often lymphocytes can be seen amongst the cells of the epithelium. In some parts of the intestinal tract, such as the tonsil or appendix, there is such a massive infiltration of lymphocytes into the overlying epithelium that its whole character is changed, and lymphocytes rather than epithelial cells form a dominant feature of the tissue.

The various formed elements, namely blood vessels, nerves and lymphatics, which are found in sub-epithelial connective tissue are to some extent concerned with the requirements of the connective tissue itself; but often they are predominantly related functionally to other associated structures. In the skin, not only

must the overlying epidermis be supplied via the dermis, but structures such as sweat glands and hair follicles, which have grown from the epithelium into the underlying connective tissue, require a rich supply of blood, as well as draining lymphatics and a nerve supply. A similar situation applies in the intestine, where in certain parts submucosal glands lie deeply in the connective tissue layers of the wall.

In many tissues, the connective elements show a different density and arrangement in different parts. Thus in the intestine there are striking differences between the more superficial subepithelial connective tissue, and that lying deeper. The former is extremely delicate, and is made up of fine reticular fibres, the usual kinds of scattered cells, and a few smooth muscle cells. This layer is continued up into the fine villous processes which increase the surface area of the intestine, and thus increase its absorptive potentiality. A small lymphatic vessel or lacteal lies in the centre of each villous process. This superficial and delicate connective tissue is separated by a thin layer of smooth muscle, called the muscularis mucosae, from the main layer of connective tissue, the submucosa. This is thicker and denser, and intervenes between the mucosa and the external layers of smooth muscle. It contains large blood vessels, lymphatics, nerve fibres and ganglion cells.

This difference in structure of the connective tissue of the villous cores and that of the submucosa, or more strikingly that of the dermis of the skin, reflects the very different function of the connective tissue in these various sites. The villi are mobile structures, concerned in the absorption of nutrient materials from the lumen of the gut, which must pass across the covering epithelium and enter the vessels lying in the delicate connective tissue of the core. The submucosa, and to a greater extent the dermis, are both supportive, and also serve as a bed in which lie the main vessels and nerves supplying more superficial structures. The submucosa forms a part of the wall of the gut, which is much more mobile than most of the skin, and is less rigid in arrangement.

CONNECTIVE TISSUE UNRELATED TO EPITHELIA

Apart from forming a basis for epithelia, connective tissue occurs widely within the animal body. Except where it is organized into specialized structures such as ligaments, tendons, or capsules for organs, its importance tends to be overlooked, and its role in the functioning of the various tissues ignored. All over the body, connective tissue has the general function of supporting and binding together the elements making up the various organs, and as should already be clear, of providing a bed in which vessels and nerves run to and from the tissues. Much connective tissue, particularly that forming a kind of packing between the various components of an organ, is relatively unspecialized; other kinds, such as bone, are organized to fulfil some particular and well defined function.

Capsules

Many organs, such as the liver and kidney, are enclosed in a fibrous capsule, which is often so well defined that it can be dissected away from the organ it encloses and studied independently. Capsules are usually made up mainly of collagen fibres and fibrocytes; extensions often penetrate into the substance of the organ in question, forming a connective tissue skeleton. Blood and lymphatic vessels, nerve fibres and ducts run in these septa, which together with the outer capsule act as a fibrous support for the cellular elements of the organ, and also as a route for essential channels of supply, excretion and control. In some organs, such as the kidney and spleen, the main vessels enter and leave the organ at one point, which can then be called the hilus; in others, such as the adrenal glands, vessels may enter and leave at various points over the surface.

The structure of such a connective tissue skeleton varies from one part of an organ to another. Thus the capsule and main penetrating septa are largely made up of collagenous fibres, but the smaller septa tend to be more delicate in structure and looser in texture, with fine reticular fibres replacing many of the coarser collagenous elements. These finer septa are often more cellular

than the larger, and the various cells previously described as occurring in connective tissue (p. 52) are found in varying numbers.

The connective tissue septa which penetrate into an organ form an integral part of it; but the outer capsule, although continuous with these septa, can often be stripped off the surface of the organ, since there is usually a well defined line of separation between collagenous tissue and the cellular tissue of the parenchyma. It is easy to remove the capsule of the kidney, for example, since provided it is not diseased, there is very little connective tissue in the parenchyma of the organ. In other instances, however, although a capsule may be well defined as a thick fibrous covering to an organ, it may be impossible to strip off without causing considerable damage to the underlying parenchyma, because of the penetrating septa. This is the situation in the adrenal gland, and in the testis, which is subdivided into lobules by septa extending from the tough fibrous capsule called the tunica albuginea.

Encapsulated structures usually lie in fatty connective tissue, which is much looser in texture than the capsule. There is continuity between outer capsular fibres and the fibres of the looser tissue in which the organ is lying, but the attachment between the two is not a firm one, and it is easy to remove the organ from its bed.

Once removed, the fatty connective tissue can be trimmed away, leaving only the capsule and the enclosed parenchyma. This trimming down to the capsule is of considerable practical importance when organs are to be weighed; but it should be remembered that it will usually be impossible to remove all traces of the loose tissue from the capsule, or to remove an equal amount from different specimens. Hence it is absurd to record the weights of dissected organs, such as adrenal glands, to fractions of a milligramme; nevertheless this is a not uncommon practice.

It is particularly difficult to appreciate some aspects of the structure of living loose connective tissue from a study of sectioned

material. The formed elements, both fibres and cells, are readily seen in such preparations, and even reticular and elastic fibres, which do not readily stain, can be demonstrated by special techniques, namely the PAS reaction, metallic impregnations, or stains for elastin. It may easily be overlooked in studying such preparations that in life the formed elements of connective tissue lie in a viscous ground substance, consisting largely of mucopolysaccharide, that is a protein-carbohydrate complex. This ground substance can be stained metachromatically; but since it shows no clearly defined structure its importance is easily ignored in sections stained by routine methods, in which it may appear only as a fine acidophilic network or coagulum. In fresh tissue, spaces in the ground substance are filled with tissue fluid, containing various dissolved salts, but these are usually extracted during the preparation of the specimen for examination.

ORGANIZED CONNECTIVE TISSUE

In many parts of the body collagen is precisely arranged to fulfil specific functions, such as the stabilization of joints, or the transmission of the forces produced by muscular contraction to the skeleton. Ligaments and capsules associated with joints are made up largely of dense collagen fibres, and are relatively acellular. The fibres are usually orientated along the lines of stress, which in narrow ligaments usually extends along their length. Tendons of insertion of muscles have the same general structure, and like ligaments these are made up largely of collagen, and contain relatively few cells. At the junctions between the actual muscle fibres and the collagenous tendon there is continuity between the tendon and the connective tissue which both encloses the muscle peripherally, and also extends into it forming septa which surrounds groups of muscle fibres (Fig. 6). Connective tissue also extends between individual fibres and forms a very delicate reticular sheath around them.

The metabolic requirements of connective tissue are relatively small, since when fully developed, as in mature tendon, it is relatively acellular. The rich vascular system present in the

submucosal layers of the intestine, or to lesser extent in the dermis of the skin, are not primarily concerned with the nutrition of connective tissue in those sites, but rather with the blood supply

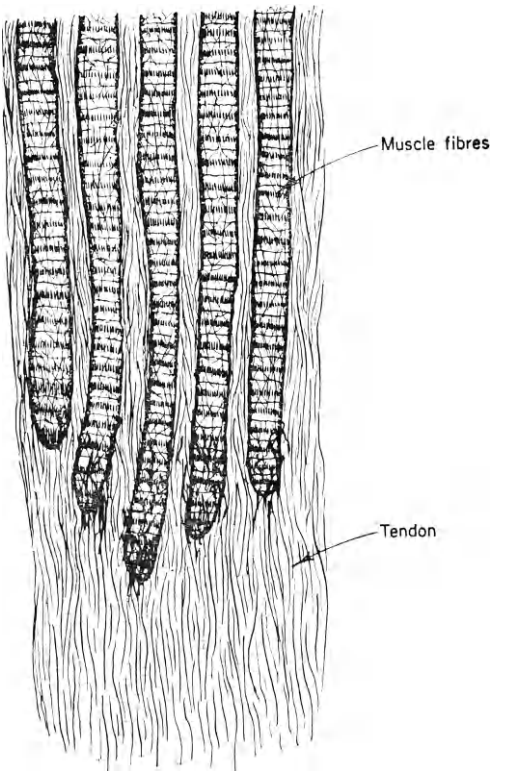


FIG. 6. The junction between muscle fibres and tendon. The muscle fibres are firmly anchored by continuity of their sheathing reticular fibres with the substance of the tendon.

to the overlying epithelium, either for the nourishment of the cells, or to take part in processes such as absorption or temperature regulation.

Cartilage

Cartilage is a connective tissue which combines firmness with a

certain amount of elasticity. The commonest type found in the body is called hyaline. In its fully developed form this consists of cartilage cells, or chondrocytes, lying in a fairly rigid matrix of collagenous fibres infiltrated with the mucopolysaccharide. The collagen present gives a certain flexibility to the cartilage, while the mucopolysaccharide gives rigidity. Despite the latter quality, cartilage contains no calcium, whose salts give rigidity to bone, and whenever appreciable quantities of calcium salts are present in cartilage, they are associated with degenerative changes. The collagen fibres in hyaline cartilage are not usually visible in histological preparations.

Cartilage is also distinguished by its avascularity and its smoothness, which combined with its strength and elasticity make it ideally suited for covering the joint surfaces of bones. The importance of the role played by hyaline cartilage in the joints is perhaps only realized when degenerative changes occur, as in various forms of arthritis. In such conditions, the smooth joint surface is eroded and roughened, and deposition of calcium converts the previously resilient covering of the bone into a harder and less flexible material. Severe limitation of movement follows these changes.

Hyaline cartilage also plays an important part in the development of the skeleton (see below). The properties of cartilage depend to a large extent on the connective tissue matrix which is infiltrated with ground substance, and two less common types of cartilage occur. The first of these is fibro-cartilage, which contains a higher proportion of collagen fibres than the hyaline variety, and is found at such sites as insertions of tendons, where rigidity and extra strength are needed. The second is elastic cartilage, which contains numbers of elastic fibres in its matrix. This occurs in places where stiffness and elasticity are required, as in the external ear.

Cartilage is easily recognizable in histological preparations from its typical structure; also it stains metachromatically (see p. 43), on account of its high content of sulphated mucopolysaccharides. If hyaline cartilage becomes involved in the process

of calcification, or in bone formation, of which calcification of cartilage is a normal step, alkaline phosphatase can be demonstrated, but otherwise this enzyme is absent.

Bone

Bone forms a major proportion of the connective tissues of the body, and provides an example of the combination of two components to give a tissue with particular mechanical properties. Bone also demonstrates structural specialization in the way it is constructed to resist or transmit certain mechanical forces.

Bone essentially consists of two major components; the first of these is an organic matrix, protein in nature, which is impregnated with inorganic salts, chiefly calcium phosphate. The organic matrix gives bone the property of resilience, while the inorganic salts give rigidity; the result is strength and resistance to deformation, but without brittleness, and with some inherent "spring". The role of the organic and inorganic components of bone can be illustrated by removing one or other of them. Immersion of a bone in dilute acid will remove the inorganic salts, but leave the fibrous part of the matrix. The bone will retain the same shape as before, but can be bent or twisted, and when released will resume its original shape. The organic part of the matrix can be removed by heating the bone in a glowing fire. Again the shape will be unchanged, but the specimen will now be extremely brittle, and can easily be broken.

The full process of the formation of bone is somewhat complex, and is described in detail elsewhere; only the basic cellular activity will be considered here.

Bone is formed by the synthetic activity of cells called osteoblasts. In most bones of the skeleton, bone is preceded by the formation of a cartilage model; but in certain sites, such as the mandible and bones of the vault of the skull, bone is laid down directly in connective tissue; this is known as "intramembranous" bone formation. The first step is the formation of pre-osseous collagen in the intercellular matrix of connective tissue. At sites where this occurs extracellular alkaline phosphatase is present,

and this enzyme may play some part in formation of the fibres (see p. 55). Osteoblasts surround the zone of fibre formation. These cells are intensely basophilic, and have a high content of RNA in their cytoplasm, an observation which suggests that they may be concerned with the elaboration of the protein component of the bone matrix. It has been shown that this basophilia reaches a peak at the time of maximum deposition of this matrix (Plate V, Fig. 18).

The next stage in the formation of bone is the infiltration of the protein matrix with calcium and phosphate salts; this constitutes the process of calcification. As the formative process continues, some of the osteoblasts, which cover the surface on which new bone is being deposited, become included within the bone. They remain there, enclosed in small lacunae, as differentiated bone cells or osteocytes. Similarly blood vessels lying in the surrounding primitive connective tissue become surrounded by bone, and remain in mature bone lying in canals. The way in which bone is laid down is reflected in its microscopic structure.

During the period of growth, bones such as those in the limbs consist of a bony shaft, the diaphysis, and two bony ends, the epiphyses. The ends usually take part in articulation in joints, and are covered with hyaline cartilage. Each epiphysis is separated from the shaft of the bone by a plate of hyaline cartilage, the epiphysial plate. This persists as long as growth continues at that end of the bone, and the proliferation of cartilage cells of this plate is the primary event in elongation of a bone. The cartilage cells then break down, and longitudinal bars of calcified cartilage extend into the diaphysis; new bone is laid down on these. At the end of the period of growth, proliferation of cartilage ceases, and the process of ossification extends through the plate, so that the epiphysis and the diaphysis are united by bone; this is fusion of the epiphysis.

Growth of bone is dependent to a large extent on the action of growth hormone secreted by the adenohypophysis, and this action of growth hormone is made use of in a technique for the bioassay of the hormone. Young rats deprived of growth hormone

by hypophysectomy are used in this procedure. After operation growth of the skeleton is greatly reduced or ceases; the epiphysial plates become narrow, and proliferation of cartilage ceases. Growth begins again if growth hormone is given to such an animal; the epiphysial plates become thicker, and active proliferation of cartilage cells of the plates occurs. The thickness of the plates in such animals is proportional to the amount of hormone given, and by comparing the thickness of the epiphysial plate of a bone such as the tibia in animals deprived of all hormone with the thickness of the same epiphysial plate in animals given preparations containing known and unknown amounts, the amount of hormone present in the unknown preparation can be calculated. This then is a method of bioassay which makes use of histological techniques.

The structure of a bone as seen with the naked eye gives striking indications of the stresses to which it was subjected during life. The human femur serves as a good illustration of this. This bone, together with that of the opposite side, receives the whole weight of the body of a standing subject, transmitted to it via the articulation of the head of the bone with the pelvis in the acetabular fossa. This force is transmitted from the head of the femur along the curved neck of the bone, and then almost vertically to the expanded lower end which forms the upper articular surface of the knee joint (Fig. 7). If a femur is sectioned along its length, differences in structure can be seen in different parts. The bone of the shaft is dense and compact, and surrounds a central cavity which is filled during life with bone marrow and fat. The upper and lower ends of the bone consist of a much thinner outer shell of compact bone, and the cavity enclosed by this is filled with a three-dimensional meshwork of slender trabeculae which constitute spongy or cancellous bone. Despite the fact that trabeculae extend in all directions, there is a marked tendency for those in the head and neck of the bone to follow an arc from the weight-bearing articular surface of the head into the almost vertically disposed compact bone of the shaft. As the shaft is followed

towards the lower end the compact bone becomes largely replaced by vertically directed trabeculae passing towards the lower articular surface; at the same time the bone expands, until

FIG. 7. Diagram of a section through the human femur, to show the orientation of trabeculae along the main lines of stress.

at the articular surface it is several times the diameter of the central part of the shaft. In a general way, the trabecular pattern follows the main lines of stress in the bone, those described being concerned in the main with weight-bearing. This, of course, is only one of the major stresses, and in life bones are exposed to a complex and variable pattern of stress resulting from muscular activity, movements and variations in posture.

The study of dried bone gives the impression that it is a static tissue. Living bone, however, is constantly changing both in form and in its actual constituents. New stresses applied to bone in a living creature will be followed by remodelling of the bone so that it can better withstand the altered forces. If a fracture has been followed by healing of the shaft at an angle, then trabeculae will develop which tend to follow the direct lines of stress along the length of the limb, rather than along the bent shaft.

There is a constant turnover of inorganic and organic constituents of bone, but the incorporation of new material in bone is, not surprisingly, greatest during the period of growth: hence the concern over the presence of radioactive strontium in the fall-out from atomic explosions, since this element is incorporated into the matrix in the same way as calcium. Moreover, bone is a vascular tissue, and any interference with the blood supply will result in necrosis of the affected part. Bone, however, has good reparative properties, provided its vascularization is good, and potentially osteogenic cells remain present within or close to bone, able to resume activity after injury.

GLANDULAR TISSUE

Glandular tissue is an organized arrangement of secretory cells for the elaboration, storage and liberation of certain products; secretory cells naturally predominate in such tissue, but their arrangement differs according to the complexity of the tissue, its mode of secretion and its situation in the animal.

The distinction between endocrine and exocrine types of secretory activity has already been pointed out (see p. 42), and it was noted that endocrine tissue passes its products directly into the bloodstream, without the interposition of a system of excretory ducts. Cells of exocrine glandular tissue may pass their products directly on to some surface; the unicellular mucus-secreting glands of the intestine are an example of this; but often a system of ducts lies between the actual secretory acini and the destination of the secretion (Fig. 8). Such ducts may be short;

sweat glands lie in the dermis of the skin, and the ducts pass through the overlying epidermis to the surface; or they may be long, as in the case of the pancreas, which lies outside the intestinal tract, but secretes into it.

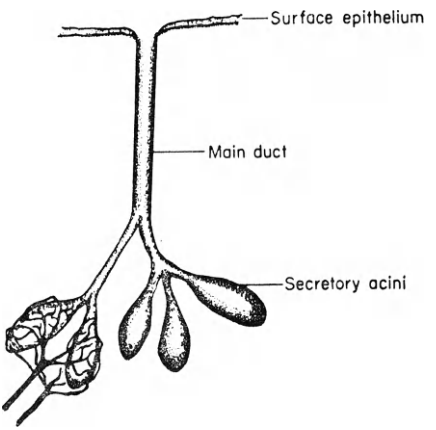


FIG. 8. Plan of an exocrine gland. Secretory acini are connected by small ducts with a main duct, which opens on to an epithelial surface. On the left, the rich blood supply to the secretory acini is indicated.

The presence or absence of ducts is therefore a major distinguishing feature between endocrine and exocrine glands; but the difference in their mode of functioning is also reflected in the organization of the secretory elements. In the majority of exocrine glands the secretory cells line blind diverticulae, which may either form long thin tubules, or more rounded structures called alveoli or acini. Glands are usually classed as tubular or alveolar according to the type of arrangement; they may consist of a single tubule or acinus, when they are called "simple"; or they may be made up of a number of tubules or acini, forming "compound" glands. The sweat glands are simple tubular in type, although the single tubule is coiled, and may be cut across a number of times in a single section. Salivary glands are examples of the compound type; in these the ducts, which must drain both

the deeply-lying as well as the more superficial secretory acini, collecting secretions from all parts of the gland and conveying them to the surface, necessarily form an extensively branched and complex system.

Endocrine glandular tissue does not show the clear arrangement of cells into secretory tubules, although during development such glands often pass through a phase during which the cells have a follicular arrangement, due to the way in which they grow from an epithelial surface. All tubular pattern has usually disappeared in mature endocrine glands, but it may occasionally persist to some extent, although any such tubules will not be connected to a system of ducts.

In other respects exocrine and endocrine glands have many features in common. The groups of secretory cells, despite their differing patterns of arrangement in the two types, in both are surrounded by connective tissue containing cells and intercellular material, and carrying blood vessels, lymphatics and nerves. Blood vessels are always present, and these are often large and numerous; lymphatics appear to be absent from some glands, such as the pituitary; and nerve fibres may either be abundant, and concerned with the regulation of secretory activity, as in the adrenal medulla, or very few, probably innervating only vessels, as in the pars distalis of the pituitary.

Glands may contain more than one kind of secretory cell. Some salivary glands are mixed in this way, so that in any one gland some of the acini may be made up entirely of cells which secrete a relatively viscid material, and are called mucous cells, while others may be made up of serous elements which stain more palely, and which secrete a more watery serous fluid. In some acini mucous and serous types of cell may both occur, and in this event the serous ones often form a cap at the blind end of the acinus.

The terminal or deepest-lying branches of the ducts of an exocrine gland are small, since they drain only one or a few acini; they are lined by a layer of flattened epithelium. As they pass towards the main ducts, the smaller ones unite with each

other and their lining epithelium becomes progressively taller, until it may be tall columnar. The thickness of the wall of the ducts also increases as the main branches are approached, largely because of an increased amount of dense connective tissue within it. The ducts themselves lie in looser connective tissue, which, extending in from the capsule, divides compound glands into lobes and smaller lobules, and provides a fibrous supporting skeleton for the secretory cells.

Secretion is an active process on the part of the acinar cell, and secretory activity will thus give rise to a pressure gradient extending from the acinar lumen towards the opening of the duct on the surface. Such a pressure gradient will tend to maintain a flow of secretions along the duct system while the gland is active. If the gland is enclosed by a capsule which is not readily distensible, any increase in the amount of blood in the vascular bed of the gland will also increase the intraglandular pressure, and may play a part in expelling secretions from the gland. The flow may be aided by the contraction of smooth muscle fibres which occur in the walls of some ducts, and also by the action of myoepithelial cells described on p. 51, which are present in relation to the acini and ducts of salivary, mammary and sweat glands. By means of their contraction these can express fluid from the acini and along the finer ducts. In the case of the mammary gland at any rate these cells are under hormonal control. When a child is put to the breast, stimulation of the nipple causes the liberation of oxytocin from the neurohypophysis. This hormone reaches the myoepithelial cells of the mammary gland via the bloodstream and causes them to contract, so that milk is actively expelled along the ducts towards the nipple.

The mammary glands provide a striking illustration of the importance of taking account of the functional state of a tissue when studying its structure. Before puberty, and even after puberty before pregnancy has occurred, the mammary gland consists largely of fibrous connective tissue, infiltrated with a variable amount of fat. A few rudimentary ducts lie in this stroma, but there are virtually no secretory acini. During pregnancy,

under the influence of the hormones circulating in the blood, a tremendous change takes place. The system of ducts extends and branches, and large numbers of secretory acini develop; the tissue becomes extremely vascular, and the stromal connective tissue largely disappears, remaining only as a delicate sheath carrying vessels and lymphatics surrounding the glandular tissue. Towards the end of pregnancy the acinar cells begin to secrete, and the acini become distended. Comparison of mammary tissue taken from a pre-pubertal or virgin animal, and from a pregnant female near term (Plate V, Figs. 19 and 20) reveal the magnitude of the structural changes associated with the onset of full secretory activity.

ENDOCRINE GLANDS

Since the secretions of endocrine glands are not passed out via a system of ducts, there is no necessity for the secretory cells to be arranged in a tubular or acinar pattern. On the other hand, the vascular system of such glands must be particularly well developed, since the cells depend on the blood not only for their supplies of essential metabolites and removal of waste products, but also for carrying their secretory products away from the gland.

Some of the endocrine glands secrete more than one active substance, and these are complex both structurally and functionally. A further complexity is introduced when endocrine cells occur associated with the same mass of tissue as cells having other functions. The pancreas, for example, contains the groups of endocrine cells known as the islets of Langerhans, which secrete the hormones, insulin and glucagon, playing a major part in carbohydrate metabolism; and also exocrine tissue which elaborates digestive enzymes which are passed into the duodenum. Examples of rather different nature are provided by the testis and ovary, which produce the male and female germ cells, but also contain endocrine cells which secrete potent male and female sex hormones.

The pituitary gland is probably structurally and functionally the most complex element of the endocrine system, and this very

complexity makes it suitable as an example of the way in which the relationship between structure and function at the cellular level can be investigated.

The pituitary gland consists of two main parts, an anterior cellular adenohypophysis, which develops in the same way as many endocrine tissues as an outgrowth from an epithelial surface, and a posterior neurohypophysis, formed by a downgrowth from the developing central nervous system. The adenohypophysis originates as an open diverticulum, known as Rathke's pouch, which grows up from the epithelium of the primitive mouth towards the brain, and comes into contact with the neural downgrowth. The pouch later becomes cut off from its origin by the formation of the base of the skull, but the posterior part of the gland retains its continuity with the nervous system throughout life by means of a stalk of nerve fibres (Fig. 9).

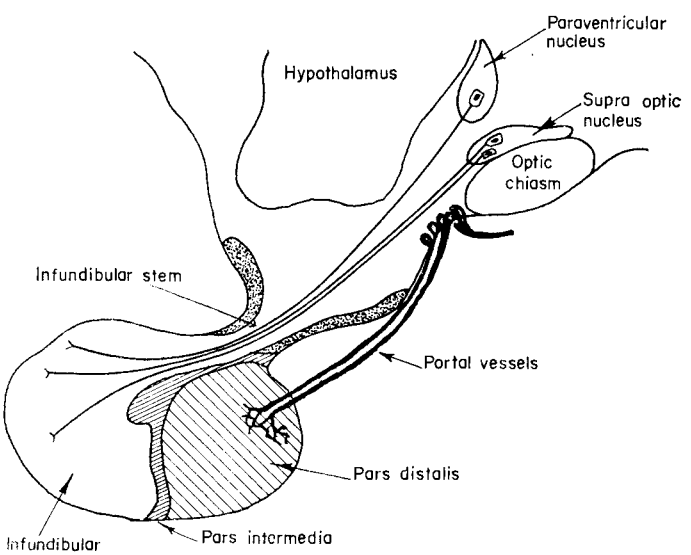


FIG. 9. Diagram of a mid-sagittal section of the pituitary gland of a monkey. The portal vessels, shown lying in front of the stalk, in fact lie superficially within it.

During the early stages of its development, the anterior part of the gland has a follicular structure, but this largely disappears by the time development is completed. During this process the adenohypophysis become differentiated into three parts. Processes of epithelial cells grow out from the main mass and embrace the stalk of nerve fibres leading to the posterior part of the gland; these form the pars tuberalis of the adenohypophysis. The original posterior wall of Rathke's pouch becomes closely applied to the lower part of the neurohypophysis, and forms a thin lamina of cells called the pars intermedia, while the largest mass of cells forms the pars distalis, which may be separated from the intermedia by a narrow cleft derived from the original lumen of Rathke's pouch. The neurohypophysis during development differentiates into three parts, an upper median eminence, which is continuous with the hypothalamus; the stalk of nerve fibres which is called the infundibular stem; and the lower expanded part of the neurohypophysis, called the infundibular process. These six distinct subdivisions constitute the mammalian pituitary gland.

Functionally, the gland is equally complex. The pars tuberalis has no known function, and the pars intermedia secretes a hormone which in lower animals controls colour of the skin. The pars distalis, however, secretes at least six hormones; one of these, somatotrophic or growth hormone (STH), governs to a large extent growth and development of the animal; another, thyrotrophic hormone (TSH), controls growth and secretion of the thyroid gland; adrenocorticotrophic hormone (ACTH) has a similar effect on the adrenal cortex; and three hormones govern reproductive activity. These are the follicle stimulating hormone (FSH) which induces development of the follicles containing ova; luteinizing hormone (LH), which together with FSH causes the shedding of ripe ova from the follicles (ovulation), and then stimulates the development of a hormone-secreting corpus luteum from the remains of the follicle; and finally prolactin, which with other active substances brings about the development of the mammary glands to a state where they are capable of the

secretion of milk. It should be noted that none of these hormones is restricted to the female, and the first two at any rate of the latter three act also on the male reproductive tract.

The adenohypophysis provides a major example of the difficulty of correlating structure and function in biological systems. In this case a primary problem is whether or not a distinct type of cell is responsible for the formation of each hormone. Initially only two types of cell were described, chromophils whose cytoplasm takes up stains, and chromophobes which stain poorly. Later the chromophils were subdivided into basophils, staining with basic dyes, and acidophils, with acid ones. As the complexity of the secretions of the gland became apparent, search was made for further cell types, which might be responsible for the elaboration of the various hormones. The use of more complex mixtures of dyes enabled other types of cell to be identified, but very often the functional significance of the colour taken up by any particular cell was ill-understood. The situation was complicated by the fact that some staining properties of pituitary (and other) cells depend on the secretory granules, while others depend on other cytoplasmic inclusions, such as the ribosomes rich in RNA already referred to (p. 37).

In recent years histochemical techniques have been applied to the pituitary, and these have made possible a more specific correlation of the staining properties of certain cells with their function, namely the elaboration of a particular hormone. The PAS technique described on p. 14 has been of great value in this respect, due to the fact that three of the hormones secreted by the pars distalis, TSH, FSH and LH are glycoproteins, and that after suitable fixation glycoprotein can be demonstrated by the PAS reaction. Many cells of the pars distalis, including those previously classed as basophils, contain PAS-positive material in their cytoplasm, and thus may elaborate and store these glycoprotein hormones.

Precise identification of the cells responsible for the elaboration of specific products, however, often entails more than simply studying stained sections of material selected at random. If we

wish to determine which cells of the pars distalis are responsible for the secretion of gonadotrophic hormones, for example, a number of lines of investigation can be followed. One of these is the study of glands of animals killed in different physiological states. Many species have a well defined breeding season. In the ferret, this extends from the spring into mid or late summer. During this time the female ferret is on heat, or in oestrus, and at this season mating and pregnancy may occur. During the winter months the animal is anoestrous, and breeding does not normally take place since the whole reproductive system is quiescent. The pituitary glands of ferrets killed during oestrus contain numerous rounded cells whose cytoplasm is filled with PAS-positive granules. During the anoestrous period such cells are few and contain few granules (Plate VI, Figs. 21 and 22). These cyclical changes in the cytology of the pars distalis correlated with active and inactive states of the reproductive system suggest that a PAS-positive cell might be associated with the elaboration of gonadotrophic hormones, which are known to regulate reproductive activity. Such a conclusion is not *necessarily* valid, however, since changes in cytology might reflect some other change in activity of the animal, such a variation in metabolism from summer to winter, or some unconnected change in other glands of the endocrine system.

Further investigation of a problem such as this might make use of experimental techniques, and in the example being considered an experiment such as that described on p. 24 might be carried out. It is known that trophic hormones secreted by the pars distalis act on other endocrine glands and stimulate them to produce hormones. FSH, which is produced by some of the PAS-positive gonadotrophic cells of the pituitary, acts on certain cells of the ovaries and cause them to secrete oestrogen, which is one of the female sex hormones. This in turn acts on the reproductive system, and initiates changes which will prepare it for the reception of a fertilized ovum, that is for pregnancy. At the same time, however, oestrogen acts back on the pituitary and inhibits the secretion of FSH, and hence that of oestrogen. This is an

example of a self-limiting "negative feedback" mechanism. Thus as the level of oestrogen rises, that of FSH starts to fall.

If, however, the target glands of FSH, namely the ovaries, are removed, no oestrogen can be secreted in response to FSH stimulation, and thus the negative feedback effect on the pituitary will be absent. As a result the production of FSH will go on increasing, as if in an attempt to stimulate the missing ovaries to produce oestrogen. The cells of the pars distalis which produce FSH become over-active, and this is associated with recognizable cytological changes in these cells. These vary in detail from one species to another, but in general they increase in size and number and become degranulated. Instead of storing a proportion of the hormone, which normally appears in stained sections in the form of PAS-positive granules, the cells secrete it into the blood stream as soon as it is formed, and appear as hypertrophied elements with only faint, or possibly absent PAS staining of their cytoplasm. In some species, such as the rat, "castration cells" appear. These are degranulated cells in which the cytoplasm is replaced by a large vesicle of colloid, very faintly PAS-positive, surrounded only by a narrow rim of normal cytoplasm in which lies the cell nucleus, displaced from its normal more central position. Although hyperactive cells of this type may be devoid of all secretory granules, a few may be seen in the Golgi zone, which is probably concerned in their elaboration.

Examination of the pituitaries of animals killed at varying times after removal of target organs such as the ovaries should enable the observer to build up a picture of the progressive changes which take place in the secretory cells of the gland before they become fully degranulated. The interpretation of the observations is however made difficult, particularly where the pituitary is concerned, by the fact that any interference with one part of the endocrine system usually affects the rest. Removal of the ovaries, for example, will also interfere with the cells of the pars distalis which secrete LH, whose target organs are also the ovaries, and these may show some cytological change which must be distinguished from that due to overaction of the

FSH-secreting cells. Endocrine glands such as the thyroid and adrenal cortex produce hormones which affect the whole metabolism of an animal, and understandably any interference with the function of these glands may cause, either directly or indirectly, some change of activity in other parts of the endocrine system. Hence, any problem such as the one discussed above must be approached from as many different directions as possible, and utilize any experimental technique which is likely to be of value. The fluorescent antibody technique, briefly described on p. 18, has already given valuable data bearing on the problem of which cells of the pars distalis produce a given hormone; but despite the great advances in staining, and the introduction of histochemical procedures, this problem is still far from solved as far as most species of animals are concerned.

In the last ten years or so electron microscopy has come to the fore in studies of the pituitary, as indeed in studies of large numbers of animal tissues. Colour and staining, in the conventional sense, play no part in this technique, but since the electron microscope can reveal structural features which lie well below the limits of resolution of the light microscope, the range of morphological features which can be used to identify various types of cells has been greatly extended. Cells of the pars distalis can be classified according to their position in the gland, their shape, and especially according to the form of their cytoplasmic inclusions, notably secretory granules (Plate VI, Fig. 23). Furthermore it is possible to make a direct correlation between the appearance of cells seen with the light and electron microscopes, since the sections used for electron microscopy are so thin that they include only a small slice of the cells sampled. It is therefore possible to cut a thin section from a block of tissue for examination with the electron microscope, and to cut the next section from the block at a greater thickness, about 1 μ or so. This section, which will include most of the cells present in the thin section, can be stained by modifications of conventional techniques, and examined by the light microscope. The thinness of the section may result in some lack of intensity of staining

compared with thicker wax-embedded sections, but nevertheless it is possible by this means to study a section passing through the same cells with both the electron and the optical microscopes. Experimental techniques, such as the ablation of target organs, can of course be used in conjunction with the electron microscope, which can be used to identify functional cell types as readily as the optical instrument.

THE NEUROHYPOPHYSIS

As already described, the neurohypophysis consists essentially of nerve fibres arising from groups of cells in the hypothalamus which pass down the infundibular stem and expand to form the infundibular process. These nerve cells elaborate two hormones, which are stored in the infundibular process, and which are closely associated with the specifically stainable neurosecretory material (NSM) (see p. 50). The electron microscope has proved particularly valuable in elucidating the precise relationship of this NSM to the nerve fibres. The fibres themselves can be stained by one of the silver impregnation techniques; NSM can be stained histochemically; but the difficulty of combining the two methods resulted in considerable uncertainty as to the precise relationship of NSM to the axon. Some thought that it formed a sheath over the surface of the nerve fibres, while others considered that it lay within the axonal membrane. The electron microscope, however, has clearly revealed not only previously unknown details of the ultrastructural organization of the neurohypophysis, and of the relationship of the nerve fibres to blood vessels, but also the fine structure of these fibres. Their membranes can be clearly demonstrated (Plate VI, Fig. 24). They enclose a number of different inclusions, among them neurofibrillae, which become sparse near the terminations of the fibres; mitochondria; small membranous vesicles, measuring about 300–500 Å in diameter, which are believed by some workers to represent the chemical transmitter acetycholine (see p. 107); and finally, dense rounded granules enclosed within membranous envelopes, usually measuring more than 1500 Å

PLATES I–IX

PLATE I.

FIG. 1. Glycogen in liver cells, stained by PAS. The fixative has displaced the glycogen towards the part of each cell lying away from the surface. ($\times$ 800).

FIG. 2. Section through the whole thickness of an artery wall. Deep to the endothelium (E) lies the muscular layer (M). Note the nuclei of the muscle cells cut transversely. Wavy elastic fibres run through the muscle. Adventitial connective tissue (A) lies outside the muscle. ($\times$ 300).

FIG. 3. Section passing tangentially through the muscular wall of an artery, and not showing any of the other layers. Note that the nuclei of the muscle cells are here cut longitudinally. ($\times$ 800).

FIG. 4. Skin of guinea-pig's ear. Note epidermis (E) and underlying dermal connective tissue (D) containing a hair follicle (F). Pigment granules (P) lie within processes of dendritic cells at the base of the epidermis, or form caps over the upper aspect of nuclei of epidermal cells. ($\times$ 500).

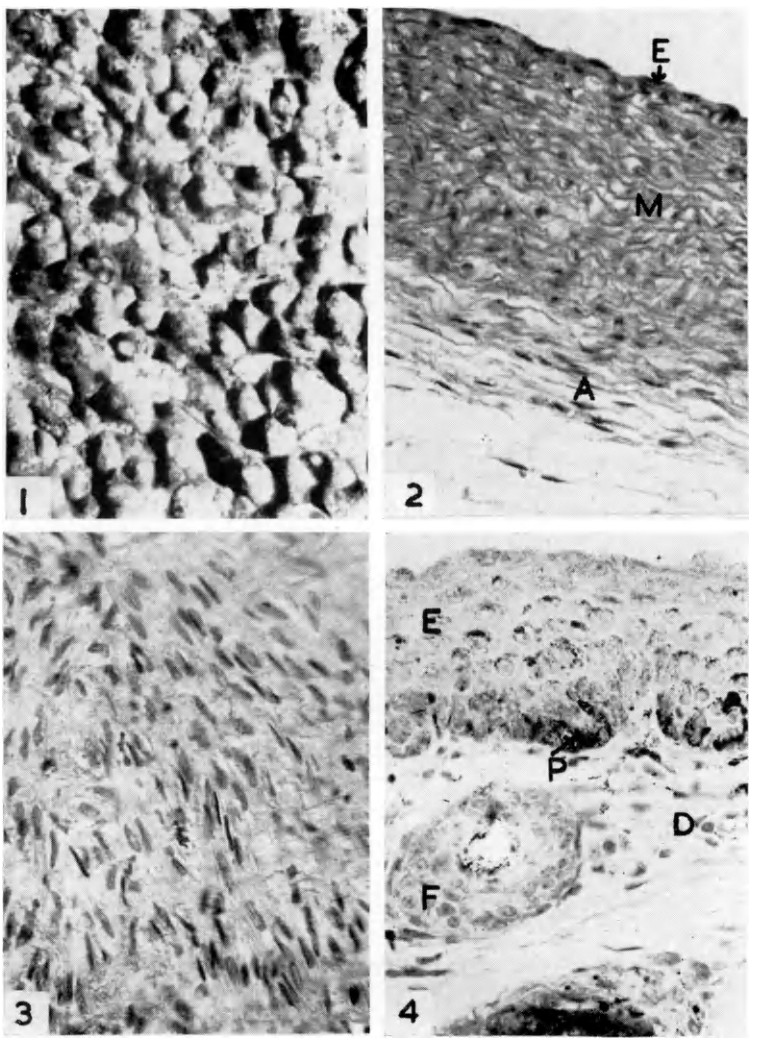


PLATE I

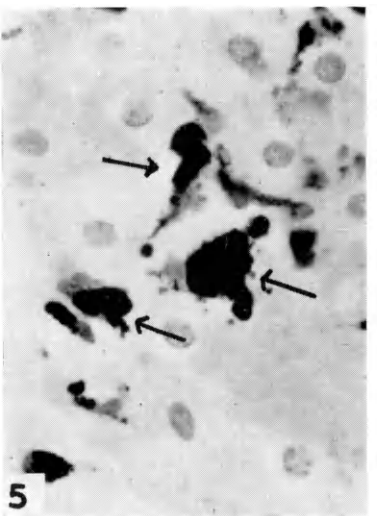
PLATE II.

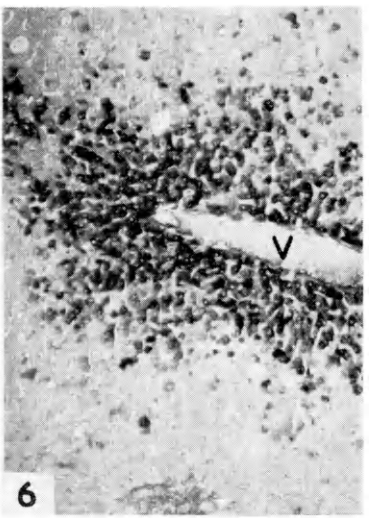
FIG. 5. Phagocytic cells (↑) in a pituitary tumour. These cells, filled with iron-rich debris of broken-down red blood cells, have been demonstrated by means of a Prussian blue reaction. (× 800).

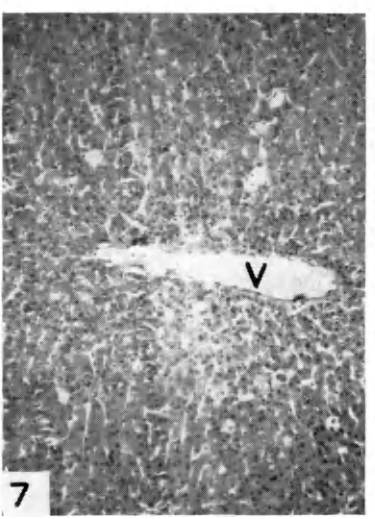
FIG. 6. Section of liver stained by PAS to show glycogen. The cells surrounding the central vein V are clearly differentiated from those further away. (× 100).

FIG. 7. A section of liver adjacent to that shown in Fig. 6, stained with haematoxylin and eosin. There is little differentiation between cells in different parts of the section. (× 100).

FIG. 8. Section of pancreas stained with methyl green-pyronin. The exocrine acinar cells (A) are deeply stained, especially basally, because of their high content of RNA. The endocrine cells of the islet of Langerhans (I) are poorly stained. (× 300).







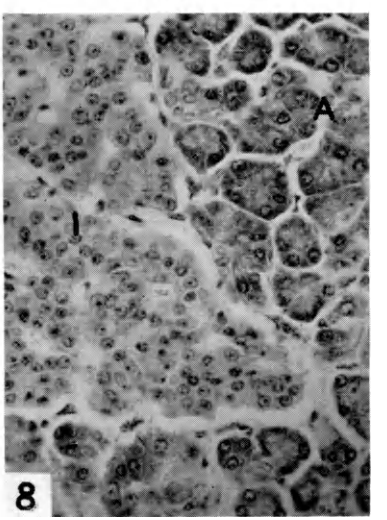


PLATE II

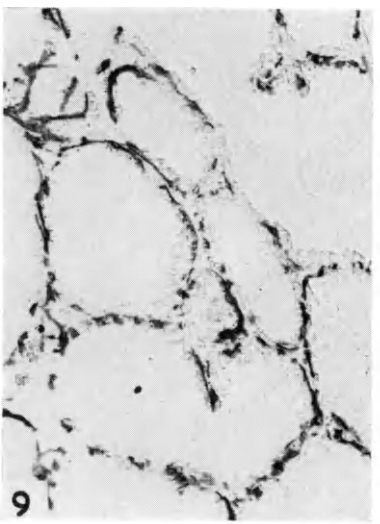
PLATE III.

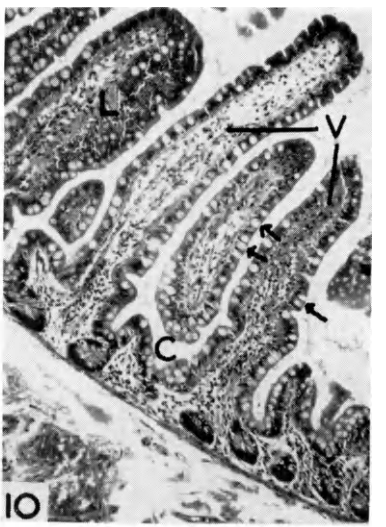
FIG. 9. Section of mammary gland from a lactating rabbit, treated to show alkaline phosphatase. The enzyme is present only in myoepithelial cells surrounding the secretory acini. (× 400).

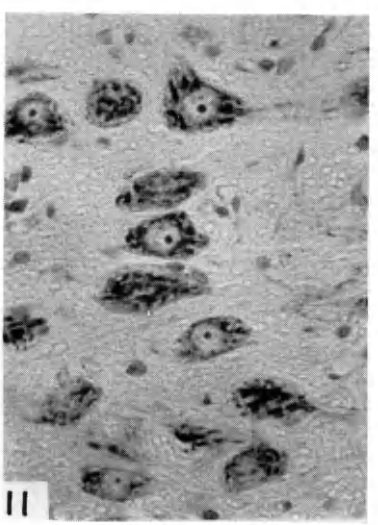
FIG. 10. Section through the wall of the small intestine. The epithelium covering the villi (V) and lining the crypts (C) contains numerous mucus-secreting cells (↑). Note the delicate connective tissue cores of the villi, and at L an accumulation of lymphocytes. (× 100).

FIG. 11. Normal motor neurones of the nucleus of the XIIth cranial nerve, stained with cresyl violet. Note the centrally placed nuclei and prominent granules of Nissl material. (× 800).

FIG. 12. Nerve cells of the corresponding nucleus on the opposite side in the same animal as in Fig. 11. The XIIth nerve had been divided seven days previously, and the neurones show marked chromatolytic changes. (× 800).







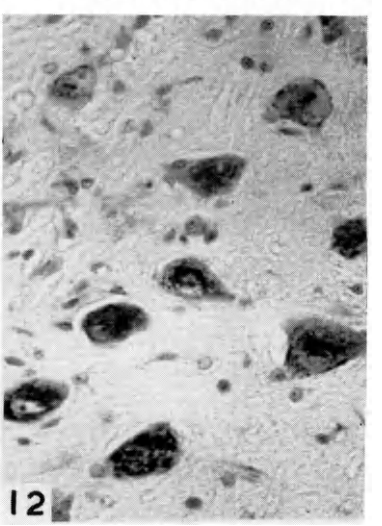


PLATE III

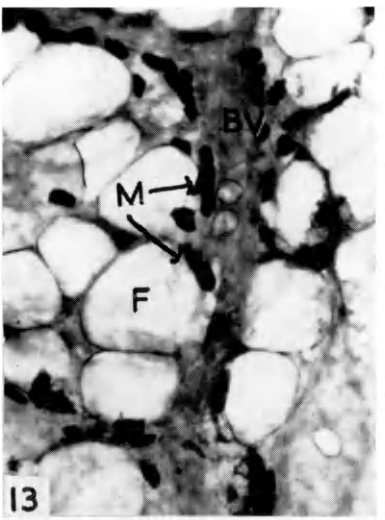
PLATE IV.

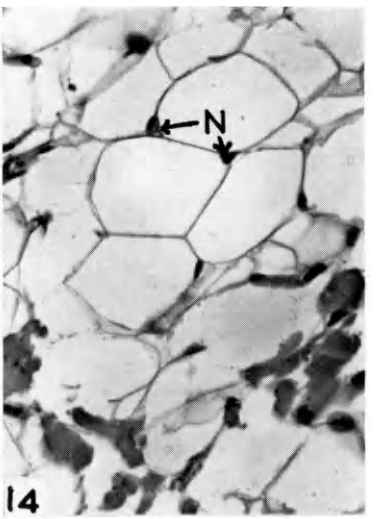
FIG. 13. Whole thickness mount of omentum from a rat's abdominal cavity. Large fat cells (F) surround a blood vessel (BV). Mast cells (M), packed with large granules which have been stained with methylene blue, lie along the vessel. (× 300).

FIG. 14. Section through a piece of fatty connective tissue. The fat has been dissolved from the cells during preparation, and "fat ghosts" remain, with nuclei (N) lying peripherally. (× 300).

FIG. 15. Section through a monkey's adrenal gland, stained with haematoxylin and eosin. C-capsule; G-zona glomerulosa; F-zona fasciculata; R-zona reticularis; M-medulla. (× 50).

FIG. 16. Frozen section of a monkey's adrenal gland, stained with Oil red 0 to demonstrate lipid, which is mainly concentrated in the zona fasciculata. (× 50).





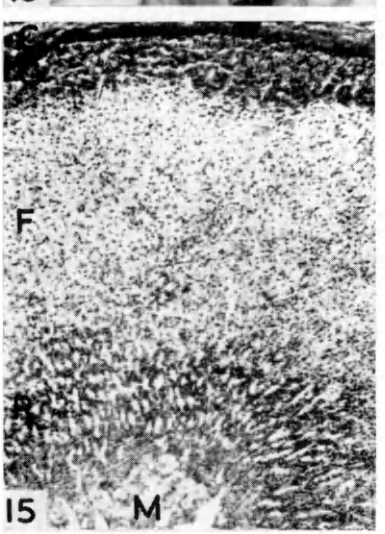


PLATE IV

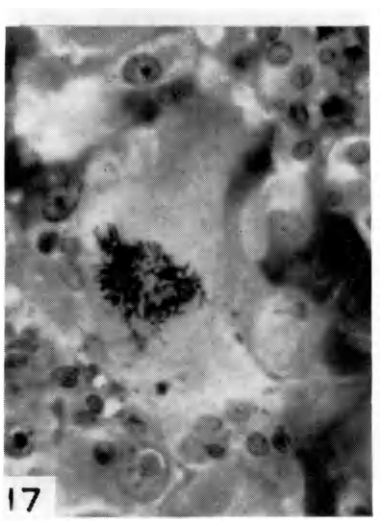
PLATE V.

FIG. 17. A mitotic figure (metaphase) in a large tumour in a rat's pituitary. The number of chromosomes is greater than in normal cells. (× 600).

FIG. 18. Developing bone in the mandibular region of a shrew. The bone (B) is surrounded by basophilic osteoblasts (O), some of which have become enclosed in the matrix. (× 400).

FIG. 19. Inactive mammary gland. A few undeveloped epithelial structures lie in dense connective tissue. (× 100).

FIG. 20. Active (lactating) mammary gland. The connective tissue has been largely replaced by secretory acini and distended ducts. (× 100).



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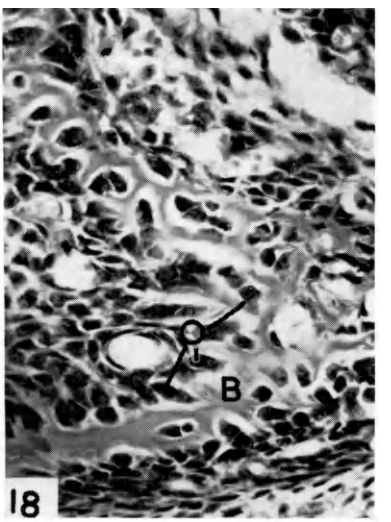

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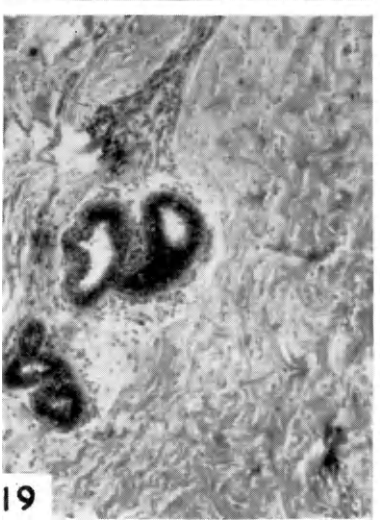

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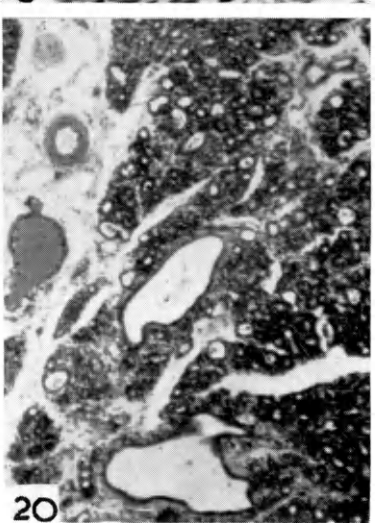

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PLATE V

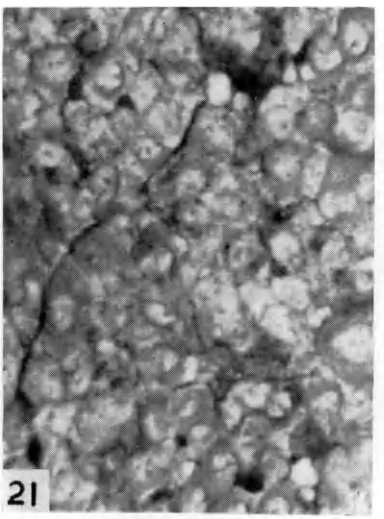
PLATE VI.

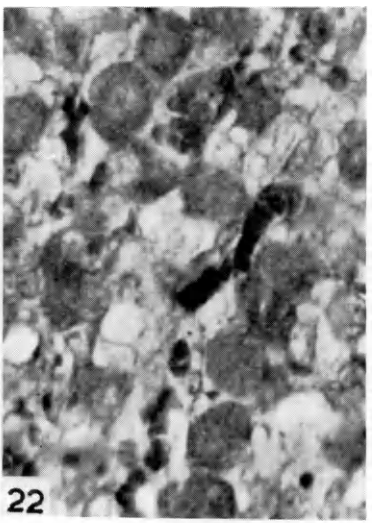
FIG. 21. The pars distalis of the pituitary gland of a female ferret during the aneostrous period. Stained by the PAS-haemalum-orange G method. (× 800).

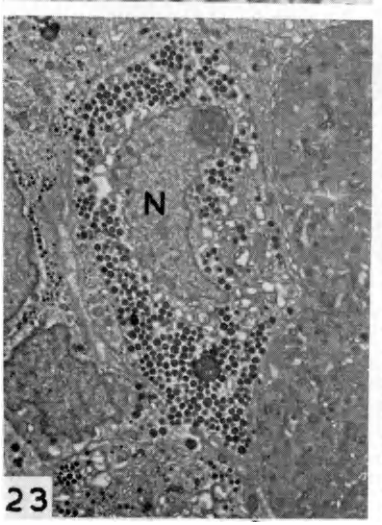
FIG. 22. Pituitary gland from a female ferret during oestrus, stained in the same way as in Fig. 21. Large round cells packed with PAS-positive granules now occur throughout the gland, and are believed to secrete gonadotrophic hormone. (× 800).

FIG. 23. Electron micrograph of an acidophil in the ferret's pituitary. The nucleus (N) is surrounded by dense secretory granules. Parts of other cells surround the acidophil. (× 4000).

FIG. 24. Electron micrograph of the infundibular process of a ferret. The figure shows parts of neurosecretory fibres (F) containing dense granular material and vesicles. These are closely applied to a pituicyte (P) which resembles a glial cell, and has watery cytoplasm. It abuts on a capillary (C), whose lumen (L) lies in the upper right-hand corner. (× 7000).







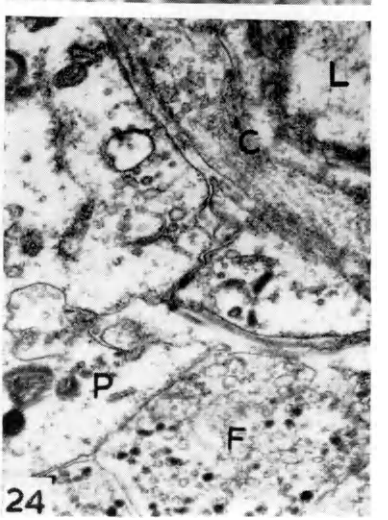


PLATE VI

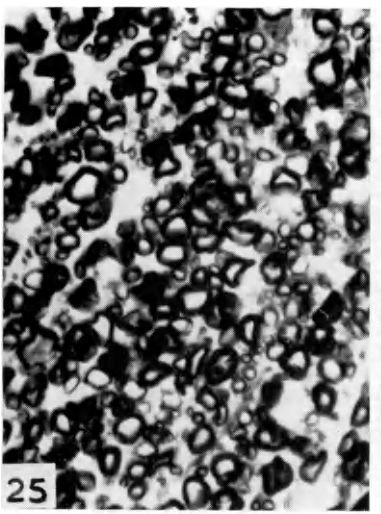
PLATE VII.

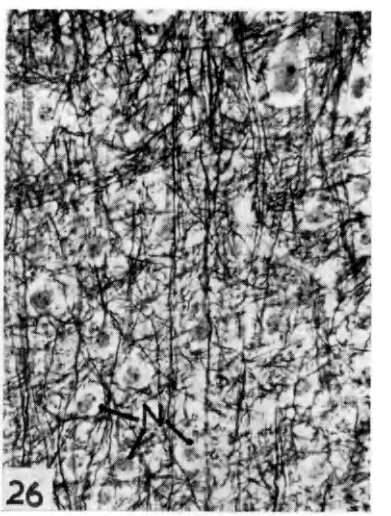
FIG. 25. Myelinated nerve fibres stained with Weigert's haematoxylin. The axons are unstained. ($\times$ 400).

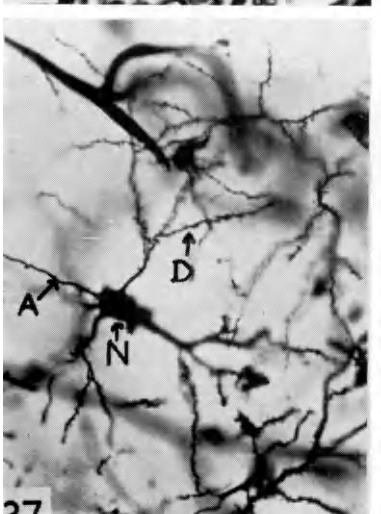
FIG. 26. Cerebral cortex, impregnated with silver to show unmyelinated nerve fibres. Neurons (N) can be seen, but show little detail. ($\times$ 600).

FIG. 27. Cerebral cortex, stained by the Golgi-Cox method. Neuronal bodies (N), dendrites (D) and an axon (A) can be seen. Some of the processes are out of the plane of focus in the thick section. ($\times$ 400).

FIG. 28. Section through the dorsal tracts of fibres of a rat's spinal cord, stained by the Marchi method for degenerating nerve fibres. The left part of the figure shows a normal tract, and the right a degenerating one. ($\times$ 75).







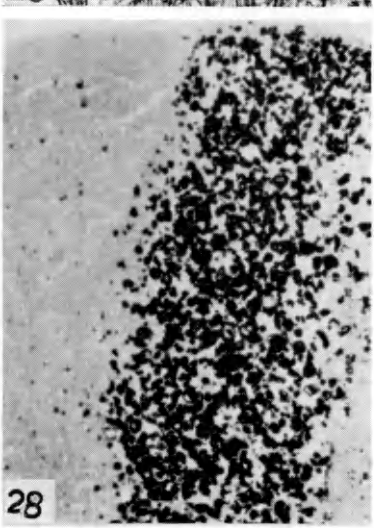


PLATE VII

PLATE VIII.

FIG. 29. Glial cells (G) in cerebral cortex, shown by metallic impregnation. At (P) a process of a glial cell forms an end foot on a capillary. ($\times$ 400).

FIG. 30. Cells of a dorsal root ganglion. Large neurones contain granules of Nissl substance, but these are finer than in motor nerve cells. Satellite cells (S) surround the neurones, and nerve fibres (N) lie above and below them. ($\times$ 600).

FIG. 31. Motor end plates on striated muscle fibres, as seen in a section, impregnated with gold chloride. Only the nervous part of the complex ending is demonstrated. ($\times$ 600).

FIG. 32. Motor end plates on rat's diaphragmatic muscle, shown by a reaction for cholinesterase. This gives a picture differing considerably from the previous one. ($\times$ 600).

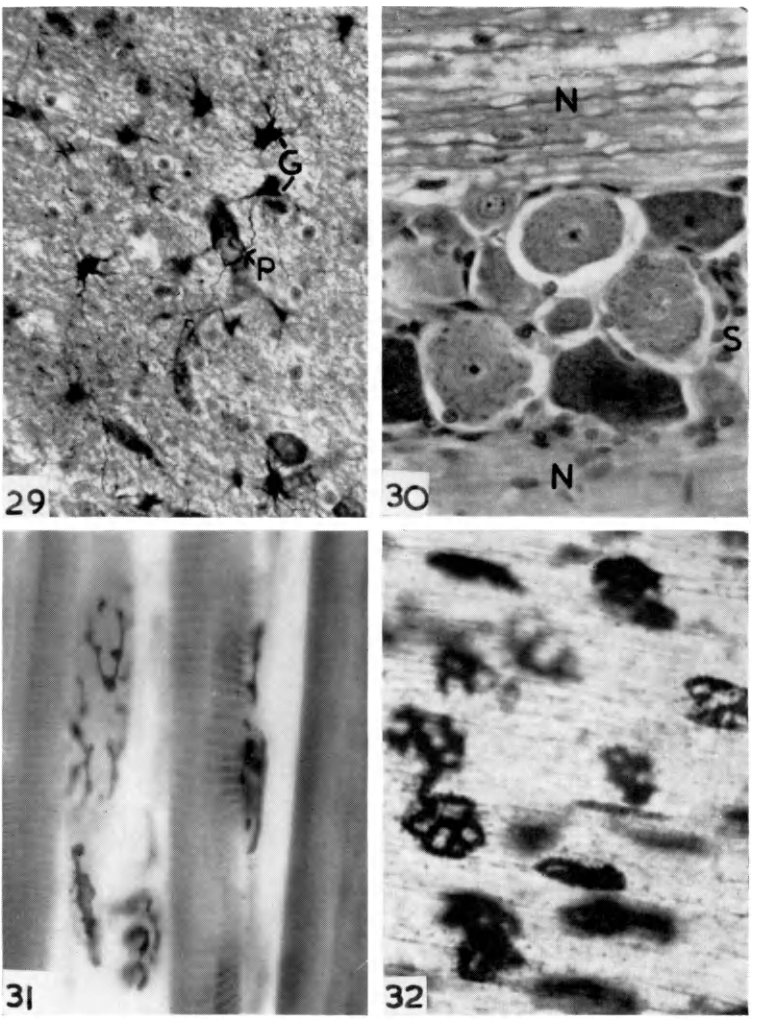


PLATE VIII

PLATE IX.

FIG. 33. Whole mount preparation of a single sensory ending in the wall of the atrium, stained by intravital methylene blue. A thick myelinated fibre (F) breaks up into numerous beaded terminals. (× 400).

FIG. 34. A similar ending to that shown in Fig. 33, as seen in a section and impregnated with silver. The main nerve fibre is seen at F, and its terminal branches at T. The picture is confused since muscle fibres (M) and connective tissue nuclei are also blackened by the silver. (× 400).

FIG. 35. A Pacinian corpuscle, as seen in section. The central nerve fibre (N) lies at the centre of a lamellated structure which is itself surrounded by dense connective tissue (CT). (× 150).

FIG. 36. Foetal skin. Note the thin epidermis, with relatively little differentiation of the various layers, and the loose underlying connective tissue. (× 100).

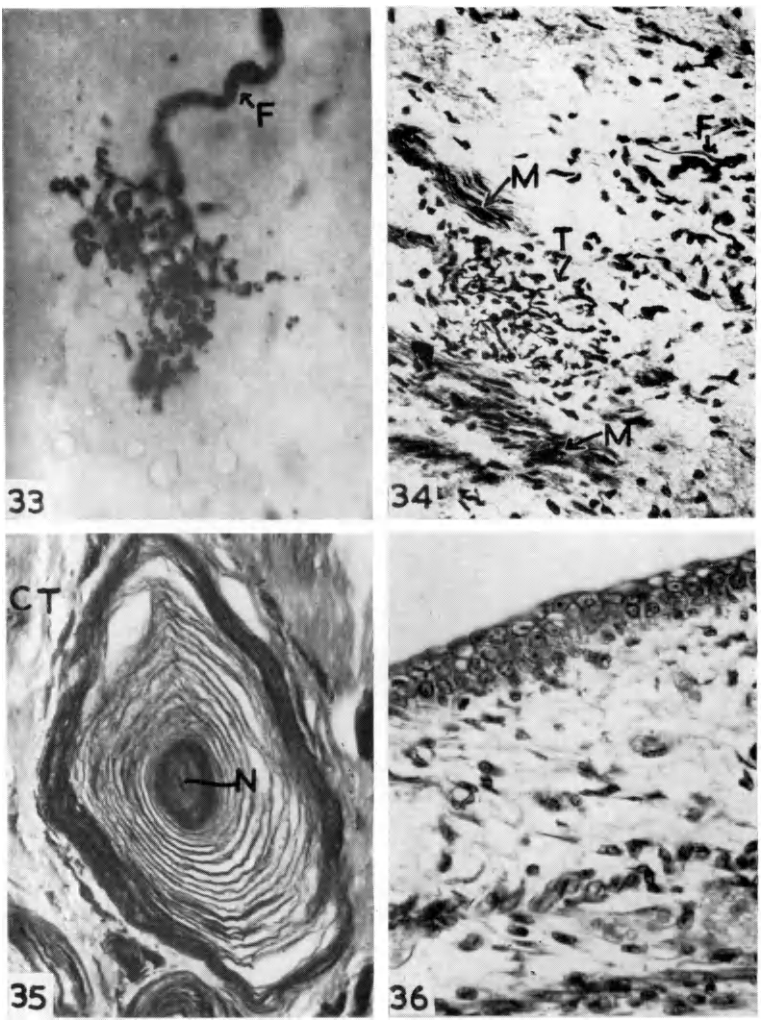


PLATE IX

in diameter. As a result of observations made both on normal material and on specimens from experimental animals it is generally agreed that the dense granules represent NSM. These granules are below the limits of resolution of the optical microscope, but stainable NSM visible in wax-embedded sections probably consists of clumps of these small granules, formed during fixation. Studies with the electron microscope have thus clearly demonstrated that NSM lies within nerve fibres, and not on their surface.

The way in which autoradiography was applied to show that NSM is synthesized in the hypothalamus, and then passes down the nerve fibres into the infundibular process, where it is stored, was described on page 23. Histochemical studies reinforce the evidence for such a mechanism. The hypothalamic neurones which elaborate the hormones have a high cytoplasmic content of RNA and acid phosphatase, which are of course associated with synthetic processes. There is little or no RNA in the infundibular process itself, suggesting that no extensive synthetic activity goes on there; but the walls of blood vessels in the process have a high content of alkaline phosphatase, an enzyme probably associated with the transfer of material, in this case the hormones, across their walls.

Experiments using tissue culture have also contributed to our knowledge of functional aspects of the neurohypophysis. Tissue from the infundibular process was grown in artificial media, and later assayed for hormonal activity. It was found that activity was present in six-day cultures, and assumed that this indicated that active hormonal synthesis by the tissue was occurring. The work was later repeated, when it was found that there was a gradual disappearance of activity, which was very slight after seven days, despite the fact that proliferation of tissue took place. Furthermore, no hormone was found in a culture made from the infundibular process of a dog which had been previously dehydrated, a procedure which is known to be followed by depletion of hormone from the neurohypophysis. It was clear following these latter experiments that the distal part of the neurohypo-

physis does not elaborate hormones in culture, and that the initial results were due to the presence of stored hormone, which was already present in the infundibular tissue before transplantation.

THE RELATIONSHIP BETWEEN THE ADENO- AND NEUROHYPOPHYSIS

It should by this time be apparent that the pituitary is a most complex organ; the way in which its activity is controlled and integrated with that of the rest of the endocrine system seems to be equally as complex as its structure. It was debated for many years whether or not secretory activity of the various cells of the pars distalis was under the direct control of the nervous system by means of nerve fibres ending in close relationship to the cells. Many papers were published which purported to show single nerve fibres, or in some cases networks, passing between the cells. It is now clear that many of these demonstrations showed not nerve but reticular fibres, which are also demonstrated by metallic impregnations such as those used to reveal nerves; and the general opinion now is that although occasional nerve fibres may be present in the pars distalis, there is in fact probably no significance secreto-motor innervation.

Despite the lack of any extensive nerve supply, however, the pituitary is very much under the control of the central nervous system, and particularly of the hypothalamus, to which the posterior part of the gland is connected by the stalk of nerve fibres from the supraoptic and paraventricular nuclei. The only direct link between the pars distalis and the nervous system is via a system of blood vessels. In many species there is no direct supply of arterial blood to the pars distalis, and this part of the gland receives the greater part of its blood from thin-walled vessels lying on the surface of the pituitary stalk. These vessels originate in the region where the stalk becomes continuous with the hypothalamus, collecting blood from a network of capillaries, called the primary plexus, lying in the nervous tissue of the median eminence. At their lower ends the vessels of the stalk empty into

the capillary plexus of the pars distalis. Since they collect blood from one capillary plexus and empty it into another they are called "portal" vessels, and are usually referred to as the pituitary or hypophysio-portal vessels.

It has been suggested that the control exerted by the nervous system over the pars distalis is exercised via these portal vessels, by means of certain "transmitter" substances, secreted by nerve fibres into the blood as it passes through the capillaries in the median eminence, which are then carried to the secretory cells of the pars distalis. Some observers consider that specific transmitter substances may be involved in such a process; but the close association between the adeno- and neurohypophysis has led others to suggest that the actual hormones of the infundibular process, known to be elaborated in the hypothalamus, might serve as transmitters. There are certainly communications between the vascular beds of the anterior and posterior parts of the gland, so that substances released from nerve fibres of the infundibular process could theoretically pass directly to the pars distalis, although as yet there is no unequivocal evidence that such a mechanism actually applies.

It is difficult to appreciate the pattern of the portal vessels and its two related capillary beds from a study of ordinary histological sections, and most of our knowledge of the anatomical arrangement of these structures has come from the application of yet another specialized technique, that of vascular injection. Indian ink, or some other opaque medium, is injected into the vessels, and the specimen fixed. The superficial pattern of the vessels can then be studied directly using a binocular microscope, or a block of tissue can be dehydrated and cleared before examination; in such a case however superposition of numerous vessels will probably obscure the fine pattern. Alternatively, the tissue can be embedded and thick sections cut (50 or 100 μ, or even more), and mounted for examination. Sections of such thickness are of little use for the study of cellular detail, but show the arrangement of the fine blood vessels clearly by virtue of their contained injection medium.

THE ADRENAL GLANDS

The adrenal glands provide another example of complex endocrine organs, but ones which are organized rather differently from the pituitary. These glands in mammals consist of an outer cortex, making up the greater part of the gland, surrounding an inner medulla. Developmentally the two parts have different origins. The cortex differentiates from the lining mesothelial cells of the primitive abdominal cavity, or coelom, near the ridges from which the gonads develop. The medullary part of the gland, however, develops from the neural crest, which lies alongside the primitive neural tube. Cells from this source migrate ventrally and enter amongst the proliferating cortical masses. Nerve fibres grow down with the neural crest elements, and eventually innervate the mature medullary cells, which developmentally correspond to post-ganglionic sympathetic neurones.

The two parts of the adrenal have very different patterns of organization. The cortex in mature mammals consists of three well defined zones of cells. Outermost is a layer arranged in loops (Fig. 10) which is called the zona glomerulosa. Deep to this is a thicker layer arranged in columns, the zona fasciculata; and between this and the medulla is the zona reticularis, in which the cells form irregular cords with no very precise pattern. Other smaller zones can be distinguished in some species. The medulla consists of islands of cells separated by connective tissue and large blood vessels.

Non-glandular tissue plays a major role in the architecture of the adrenal. The gland is enclosed in a capsule of dense collagen, which merges with the looser fatty tissue in which the glands are lying. Arnold in 1866 showed how the connective tissue of the capsule penetrates into the cortex and forms a reticular basket around the cells of the zona glomerulosa. Long septa extend deeply from this zone between the cells of the inner two zones, the pattern corresponding to the arrangement of the cells. These septa of collagen carry the blood vessels, which pass in to the medulla through the cortex. The blood vessels can be seen to

form a prominent feature in injected preparations; but in sections not specially prepared to demonstrate them, they are often collapsed and relatively inconspicuous, except in the medulla.

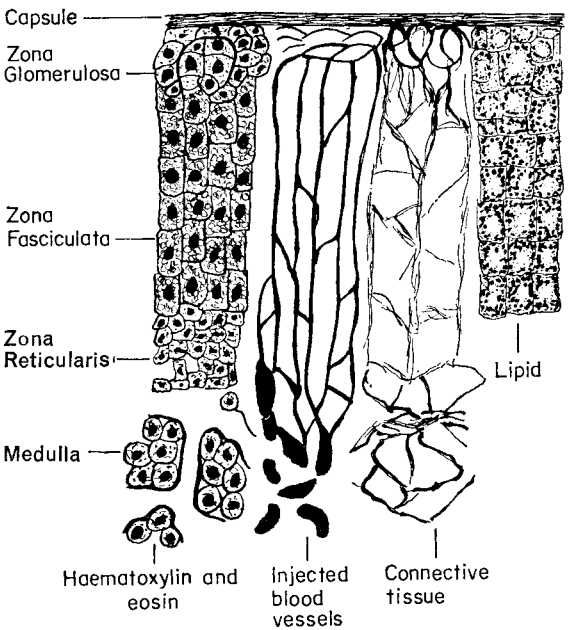


FIG. 10. Schematic section through part of an adrenal gland, to show the various component tissues and cells.

The cells of the cortex vary in structure in the different zones. Those of the zona glomerulosa are of medium size and often appear somewhat compressed. In the zona fasciculata the cells are large and cuboidal with centrally placed nuclei; while those of the zona reticularis are usually rather small, and their cytoplasm stains strongly with acid dyes. They are sometimes referred to as "compact" cells; some of the cells in this inner zone appear to be degenerate.

Frozen sections stained to demonstrate lipid show that, in many species, the cortex contains an abundance of this material,

although the glands of ruminants such as the cow and the sheep contain little. The distribution of the lipid varies from one species to another, but usually the cells of the zona fasciculata are filled with droplets, while those of the zona glomerulosa contain less, and those of the zona reticularis are either devoid of lipid, or contain very little.

The significance of the zonation of the adrenal cortex has attracted the attention of many research workers. It has been widely suggested that the innermost layers of cells, forming the zona reticularis, consist largely of degenerating elements, and that new cells formed in the outer layers of the cortex gradually migrate towards the medulla. Some weight was given to this view by the finding that if the gland is enucleated by slitting the capsule and squeezing out the contents, a complete three-layered cortex can regenerate from the few cells of the zona glomerulosa which are left adhering to the capsule. Recently studies using autoradiographic techniques have confirmed that migration of cells does occur. As already noted on p. 22, cells can be labelled by injecting tritiated thymidine, that is thymidine containing the radioactive isotope of hydrogen, tritium, into a living animal. This material is incorporated into the DNA of mitotically active cells, which thus become tagged. If such a procedure is carried out using mice, and the adrenal glands are removed from the animal a few days after the injection, labelled cells are found in the outer layers of the adrenal cortex; but if glands are obtained from animals that were allowed to live for some weeks after injection, labelled cells are found in the inner cortical layers. This observation suggests that cells migrate from the outer to the inner layers of the cortex in the course of a few weeks. Similar results have been obtained from experiments using rats.

As is often the case where biological problems are concerned, however, there is not yet any definite answer to the question as to whether the zona reticularis is degenerate or not. Degenerate cells would be expected to be less active than healthy ones; and yet histochemical studies have shown that the zona reticularis contains a high concentration of RNA, phosphatase and succinic

dehydrogenase. The latter enzyme is associated with a series of oxidative reactions playing an essential part in cellular metabolism, known as the Krebs cycle. The presence of substances usually associated with metabolic activity perhaps suggests that the zona reticularis may contain metabolically active cells (see below).

The adrenal cortex secretes a number of different hormones which constitute a group of chemically related steroids. Some of these affect the metabolism of carbohydrate, fat and protein; others influence mainly the excretion and resorption of electrolytes; and some have properties of male and female sex hormones. One of the cortical steriods, called aldosterone, is particularly potent in causing the retention of sodium by the body.

Many studies of the adrenal have been concentrated in the investigation of the role of the various zones of the cortex in the elaboration and secretion of these various hormones. Attempts to localize the sites of formation of these substances have made use of histochemical techniques. The lipid material, which as already noted occurs in large amounts in cortical cells of many species, has naturally been widely studied. It differs from ordinary fat in that it is birefringent, and contains large amounts of esters of cholesterol, a substance whose molecular structure is very similar to that of the steroids. The path of biosynthesis of steroids can indeed be traced through cholesterol.

It certainly appears likely that there is some relationship between lipid and the cortical hormones, and the cortex becomes depleted of lipid when an animal is stressed, a procedure which results in the outpouring of ACTH by the pituitary, and the subsequent release of large amounts of cortical hormones from the adrenal. Histochemical demonstration of the cortical hormones has not, however, been possible up to the present, although a number of attempts have been made. The steroid hormones contain ketonic (C=O) groups, and also have strong reducing properties, and these chemical characteristics have been made use of in an attempt to devise a suitable histochemical method for their demonstration, so far without any striking success. It must

be remembered, however, that the active substances will be present in the cells in only extremely small amounts, which may preclude their demonstration under the microscope.

Removal of the pituitary gland from an animal is followed by profound histological changes in the adrenal cortex as a consequence of the loss of ACTH. The two inner zones, fasciculata and reticularis, become shrunken, but there is little change in the zona glomerulosa. At the same time there is a great reduction in the secretion of cortical steroids, with the exception of aldosterone, which appears to be little affected. Such experiments thus seem to indicate that the zona glomerulosa is responsible for the secretion of the latter hormone, and that the two inner zones elaborate the other corticoids. They further indicate that the outer zone of the cortex is less under the influence of the trophic hormone of the pars distalis, which seems to exert its effect mainly on the inner cortical layers.

The question of the function of the zona reticularis has already been raised, with reference to the suggestion that cells of this zone are degenerate. An alternative view is that these cells are in fact extremely active in the production of hormones, at any rate in some species, among them the human. It has been observed that the administration of ACTH, or subjection of an animal to prolonged stress, which results in release of this substance from the pituitary, is followed by changes in the cells of the zona fasciculata. These cells usually have cytoplasm which stains lightly; but after such stress, they become denser and more compact, and resemble cells of the zona reticularis. This morphological change is accompanied by an increase in the amount of phosphatase, succinic dehydrogenase and RNA, which can be demonstrated histochemically in the cells. It has been suggested that these changes are associated with the increased production of steroid hormones which is brought about by the action of ACTH on the cortex. More than this, it is claimed that the cortical hormones, with the exception of aldosterone, are normally produced by cells of the zona reticularis, and that the zona fasciculata is made up of cells storing the precursors of these

hormones. In times of need, under the influence of ACTH, the zona fasciculata begins to manufacture these hormones itself, so that instead of acting as a store for precursors, it comes to resemble the zona reticularis functionally as well as morphologically.

The vascular pattern of the adrenal has already been briefly described. In some species the vessels show particular features which may play a part in the regulation of the gland's activity. In the human, the venous blood from the gland is drained away by means of one large and several smaller accessory veins. The wall of the large vein contains thick longitudinal bundles of muscle, and capillaries draining blood from the cortex open into the main veins only after passing between these bundles. Contraction of the muscle fibres could thus diminish the amount of blood draining from the cortex, and by slowing the blood flow could as a secondary effect reduce the supply of oxygen and metabolites to the cortical cells. In this way the activity of the cells might be affected, and some change in the production of steroids occur as a consequence.

As in the case of the pars distalis of the pituitary, it seems unlikely that a direct innervation plays any major part in regulating the activity of the adrenal cortex. Nerve fibres can readily be demonstrated in the capsule and between the cortical cells, both by classical silver impregnation techniques, and also by histochemical methods for showing cholinesterase. It seems likely, however, that any nerves in the cortex are either concerned with the innervation of blood vessels, or are passing through to the medullary part of the gland.

ADRENAL MEDULLA

As already described, the adrenal medulla develops from an entirely different source from that of the cortex, and is formed by migrated neural crest cells. The medullary cells are developmentally equivalent to the sympathetic neurones which lie in the paravertebral ganglia of the autonomic nervous system. As they migrate to the site of the developing adrenal, they are accompanied by nerve fibres, which end on the medullary cells and constitute a

true secreto-motor innervation. Stimulation of these nerve fibres is followed by release of the medullary hormones.

The cells of the adrenal medulla produce two hormones, adrenaline and noradrenaline. Noradrenaline is also secreted at the endings of sympathetic nerve fibres. It is mainly responsible for the maintenance of vascular tone, and is liberated in response to the need for circulatory adjustments. Adrenaline, while sharing certain properties with noradrenaline, is of primary importance in regulating the regional blood supply to certain organs, and in bringing about certain metabolic adjustments.

The cells of the adrenal medulla are part of a widely distributed system of "chromaffine" tissue, made up of cells that become deeply pigmented after treatment with chromium salts such as potassium dichromate. The adrenal medulla forms the largest collection of such cells, but numerous smaller groups occur throughout the body, particularly in relation to the great blood vessels and dorsal abdominal wall.

A positive chromaffine reaction is a characteristic of all medullary cells; but other histochemical techniques enable two types of cell to be distinguished, corresponding to secretors of the two medullary hormones. One method is to treat fresh slices of adrenal tissue with a solution of potassium iodate for about 12 hr, at a pH of between 5 and 6. Dark pigment develops in some medullary cells after such treatment, and these are the cells containing noradrenaline. Pigment fails to develop during this time in cells containing adrenaline. Cells containing noradrenaline also fluoresce strongly when illuminated by ultraviolet light, while cells containing adrenaline fluoresce only weakly. The two types of cell can also be distinguished using the electron microscope.

Medullary cells have some regularity of arrangement relative to the blood vessels, and in some species of mammals there is a definite polarization of the cells. In the cat, for example, they have an arterial and a venous pole, the arterial one being applied to a capillary, the venous one to a vein. Release of secretory material takes place directly into the venous blood.

In lower vertebrates there are no adrenal glands of the form described above. Instead diffuse masses of "interrenal tissue", equivalent to the cortical tissue of mammals, lie between the kidneys, mingled with scattered collections of chromaffine cells. The two types of secretory tissue forming one composite gland in higher vertebrates raises some speculation as to whether the association has any functional significance; but so far no clear evidence for this has been found.

THE ORGANIZATION OF NERVOUS TISSUES

The nervous system is concerned both with the regulation of internal activity of the organism and with the mediation of environmental influences on this activity. Nervous activity can be broadly classified as "integrative", but the various parts of the nervous system differ widely in their precise function. Functionally we can separate nervous activity, and structurally, the nervous system, into an afferent or input part; an efferent or output division; and in between these two, centres of correlation, concerned with the integration of the complex input of diverse impulses, the effecting of the requisite adjustment of the internal milieu, and the appropriate reaction of the animal in relation to its environment. Complex in its activity, the nervous system is also complex in organization; nevertheless there is a basic structural pattern, which is modified regionally in accordance with functional specialization.

METHODS OF STUDY OF THE NERVOUS SYSTEM

The majority of routine histological techniques have only limited value when applied to nervous tissue, and in order to gain a full appreciation of its organization, a number of specialized techniques must be used. Since understanding of functional organization must be based largely on an understanding of structural aspects, it is appropriate to consider these specialized techniques before dealing with some aspects of the organization of nervous tissue.

Basic dyes will stain both the nuclei and the cytoplasmic Nissl

material of nerve cells, and as already noted on p. 47 a particular group of analine dyes is often used for this purpose; the members of this group are called collectively Nissl stains, and include cresyl violet and toluidine blue. These stains give a particularly clear picture of the amount and distribution of Nissl substance in the cell, and will also demonstrate any abnormalities, such as the changes associated with chromatolysis.

Nissl stains, however, will not stain other structures in the nervous system, apart from nuclei of non-nervous cells such as the supporting neuroglia, or the endothelial lining of blood vessels. The sheath of lipo-protein, myelin, which invests the larger nerve fibres, can be stained by dyes used to demonstrate fat, such as Sudan black, but only so long as the tissue is not exposed to fat solvents during its preparation. A commonly used method which avoids the complication of cutting frozen sections is that of using a special haematoxylin after preliminary treatment of the specimen with a mordant containing potassium dichromate. After embedding in paraffin and sectioning, the sections can be stained according to the usual method for wax-embedded sections, overstaining with the haematoxylin and then differentiating until only the bundles of myelinated fibres retain the dye. This is Weigert's method, and it remains the most commonly used technique for showing myelinated tracts, which are coloured bluish-black (Plate VII, Fig. 25). Cells are, however, unstained. A more recent technique, also used with wax-embedded material, uses the stain Luxol fast blue, which stains the neurokeratin component of the myelin a bright blue colour. This latter method has the advantage that a counterstain can then be used to demonstrate the cells, so that cells and myelinated fibres can be studied in the same sections.

Neither the Weigert nor the Luxol fast blue techniques stain the finer unmyelinated nerve fibres, since they colour only the sheath, and not the axonal material. To demonstrate this, one of the metallic impregnation methods must be used. A number of techniques were introduced by Cajal, adapted to tissues from different parts of the nervous system, and varied also according

to the age of the animal from which the tissue was obtained. The methods depend on the impregnation of the material with silver nitrate, either in block, or as sections, followed by treatment with photographic developers. Neurofibrils of the neurone and its processes become blackened due to the deposition of a reduced silver compound on their surface, and result in preparations which when suitably mounted as sections can be studied with low or high powers of the microscope (Plate VII, Fig. 26). Variants of such metallic impregnation techniques are also used to demonstrate the small terminal swellings of axonal branches by means of which functional contact is made with the dendrites or cell bodies of other neurones.

One of the difficulties of studying the relationships of cells and fibres in nervous tissue is that the processes of neurones extend three-dimensionally to a greater or lesser degree. It is impossible to determine the full extent of the processes of any given cell in thin sections, since many of them are likely to extend beyond the thin slice of tissue included in the section. Thick sections stained to show fibres will help little, since the enormous numbers of cells and processes make it impossible to follow those of individual cells for any distance. Fortunately, however, there is a technique which enables us to study the whole neurone and its processes. This is the Golgi, or Golgi-Cox technique, named after the histologist Golgi who discovered it, and after Cox who modified the original method. The technique depends on the fact that if small pieces of nervous tissue are immersed for a long period in a solution of dichromate, or of dichromate and osmium tetroxide, and then treated with silver nitrate, a small proportion of the neurones present are stained intensely black over their whole extent, so that cell body, axon and dendrites are all coloured. If thick sections, of the order of 200 μ or so are cut, dehydrated, cleared and mounted, then the branches of the various processes can be followed by focussing up and down with the microscope. The great value of this technique depends on the fact that only a few neurones in the tissue are blackened, so that these stand out clearly (Plate VII, Fig. 27); if all were coloured, such a thick

section would present an impenetrable mass of superimposed cells and fibres, and no detail of the extent of individual elements would be visible. The reason for the selective staining of only a few cells is not known, but it may depend on their functional state at the time of fixation, or possibly on other unknown factors.

Another technique, which has considerable value in the study of certain types of nervous tissue, is that of intravital staining. This depends on the fact that living nerve fibres will selectively take up the dye methylene blue from a very weak solution and become stained, whereas most other tissues will not. The technique is to inject a dilute solution of the dye into the bloodstream of a living anaesthetized animal. After some 20–30 min the animal is killed, and tissues removed and placed for a further period in a dilute solution of the dye. They can then be mounted for examination, with or without some form of fixation. This method is particularly useful for demonstrating nerve fibres and endings in tissues which are readily exposed to the dye circulating in the blood, such as those lying in the wall of the heart and great vessels (see Plate 9, Fig. 33).

Much of our understanding of the organization of the nervous system has come from studies of the results of experimental interference which results in both structural and functional changes. The origin, course and destination of tracts of nerve fibres can be determined either by destruction of a group of nerve cells, which will be followed by degeneration of the processes arising from such cells, or by cutting the tract of fibres at some point in their course. In the latter case, fibres distal to the cut will degenerate, while the cells giving origin to them will show a greater or lesser degree of chromatolytic change. Methods of staining already described may be used to demonstrate such changes; Nissl stains, as already described (see p. 47) will reveal the loss of Nissl material which accompanies chromatolysis, and if the damage is sufficiently severe the affected cells will die, and be replaced by glial cells, a process called gliosis. Degeneration of a tract of myelinated fibres will be accompanied by breakdown and eventual disappearance of the myelin, so that an unstained

area will be left, which may contrast with a normally stained tract on the opposite side. If the affected fibres are not closely grouped together in a single tract, however, their disappearance might not be readily seen, and in such a case a method intended to demonstrate degenerating fibres in a more positive way might be used, such as the Marchi technique.

This technique depends on the fact that degeneration of myelinated axons is accompanied by changes both in the form and chemical properties of the myelin. The sheath breaks up into globules, and oleic acid is formed. If a piece of nervous tissue containing both normal and degenerating myelin is fixed in a mixture containing potassium dichromate and osmium tetroxide, the normal myelin is oxidized by the dichromate and as a result it is not stained by the osmium tetroxide. Oleic acid, however, stains readily with the osmium, so that the course of degenerating myelinated fibres will show as a line of black globules against a background of unblackened normal myelin (Plate VII, Fig. 28). This technique has however certain disadvantages; it can only be used within a certain time of making the lesion; if applied too soon (within the first week) there will not yet be enough degenerative products to stain; and if applied too late such products have been largely absorbed. Furthermore, false positive reactions sometimes occur, so that some care must be used in interpreting the results.

The Marchi method clearly cannot be used for the study of degenerating unmyelinated fibres. Absence of these might be shown in sections stained by one of the metallic impregnation techniques; but methods have also been developed to demonstrate the fragmentation of such fine fibres, and the swelling and disintegration of their terminal swellings during the course of their degeneration.

DEMONSTRATION OF NON-NERVOUS TISSUES

Although neurones and their processes make up a large part of the nervous system, other elements play an essential part in its functioning, and some of these can only be demonstrated by

special techniques. The membranes enclosing the central nervous system, namely the tough fibrous dura mater forming the outer layer, and the more delicate inner pia-arachnoid, can be stained by the usual histological techniques. Similarly, the fibrous sheaths of the peripheral nerves can be coloured without recourse to special stains. The cellular supporting tissue of the central nervous system, the neuroglia, however, requires other methods. These cells, whose disposition will be discussed later, are usually demonstrated by some kind of metallic impregnation technique employing salts of silver or gold (Plate VIII, Fig. 29). In some cases special stains, such as one called phosphotungstic acid-haematoxylin, can be used.

Blood vessels form an important component of most tissues, but particularly of those of the nervous system, whose cells can survive lack of oxygen for only a very limited time. The structure of vessels of the nervous system can be studied by the usual histological methods; but the general pattern of the arrangement of the finer branches must be examined in tissue in which the vascular tree has been injected with some opaque medium such as indian ink, or coloured gelatine. The pattern of the vessels can then be studied in thick sections, which are cleared and mounted for microscopic examination. Another method, which has been particularly applied to the nervous system, is that of staining the blood already present in capillaries, by means of a reaction which forms the basis of a test for the demonstration of haemoglobin. This, called the Pickworth technique, can give a clear picture of the disposition of capillaries in thick sections of the nervous system, provided of course that these were filled with blood at the time of fixation of the tissue.

THE ORGANIZATION OF THE NERVOUS SYSTEM

It is convenient to divide the nervous system into central and peripheral parts, the central being concerned to a large extent with integrative activity, the peripheral with the reception of stimuli arising from the creature's internal or external environment, and the transmission of nervous impulses to and from the

central nervous system. Environmental stimuli give rise to nervous impulses at the point where they impinge on some sensory receptor structure; these impulses are conducted to the central parts of the system, and from this, as a result of the interaction of various neuronal "circuits", efferent impulses pass out to the appropriate effector tissue.

The general characteristics of the neurone, which constitutes the key element of the nervous system, have already been considered. But equally as important in nervous activity are the synapses, or junctional zones between one neurone and another, across which nervous impulses are transmitted from cell to cell. The synaptic swellings, or boutons, are formed as enlargements at the ends of fine terminal axonal branches. They lie closely applied to the cell body or to dendritic or sometimes axonal processes of other neurones. They are usually about 1–2 μ in

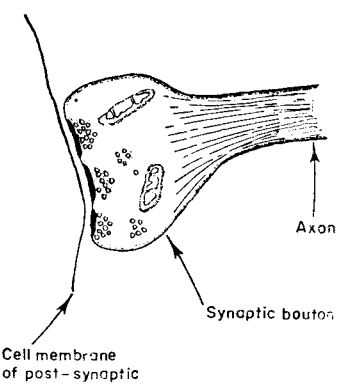


FIG. 11. Diagram of a single synaptic terminal, as seen with the electron microscope. The bouton contains small synaptic vesicles and mitochondria.

diameter, but may be larger, and it has been estimated that more than 2000 such endings, at the ends of branches of many different axones, may be found on a single large motor nerve cell, such as a

motor neurone in the spinal cord. Boutons may cover 4/5 of the surface of such a cell.

Light microscopy reveals little of the internal structure of synaptic boutons, although they can be demonstrated by metallic impregnations, and also by certain methods for the staining of mitochondria. The electron microscope has shown that they are in fact rich in mitochondria, and also contain large numbers of small membranous structures, measuring about 300–500 Å in diameter, which have been called synaptic vesicles. There is some evidence that these latter structures may be associated with one of the chemical transmitter substances of the nervous system, acetylcholine. Electron micrographs have also shown that the boutons are not quite in contact with the membrane of the neurone on which they lie, but are separated from this (the sub-synaptic membrane) by a narrow gap (Fig. 11).

PERIPHERAL NERVES

The simplest part of the nervous system as far as structure is concerned consists of the peripheral nerves, such as can be dissected in the limbs. These are made up to a large extent of motor and sensory processes of neurones, bound together by connective tissue. It should already be clear, however, that even in the peripheral nerves there is a good deal of complexity, since the processes of the neurones are surrounded by the neurilemmal sheath and in many cases by myelin, and that this sheath in turn is enclosed in a layer of reticular fibres. This reticular sheath is continuous with a somewhat coarser sheath binding together bundles of nerve fibres, and finally with an outer sheath which surrounds the smaller bundles. This connective tissue both acts as a binding material, and also serves as a bed for the blood vessels which form longitudinal channels along the nerve.

The fibres from which the peripheral nerves of the body are derived arise segmentally from the spinal cord by means of dorsal and ventral roots. The diagram of a cross section of the spinal cord, passing through a pair of such roots (Fig. 12)

illustrates how these are arranged, and where the motor and sensory neurones, from which these fibres arise, are lying in relation to the cord.

Sensory fibres of the spinal nerves, which conduct impulses into the central nervous system, are called afferent, and come from cells lying on the dorsal or sensory roots of the nerves which form the dorsal root ganglia. The motor or efferent cells lie within the central nervous system, in the ventral part

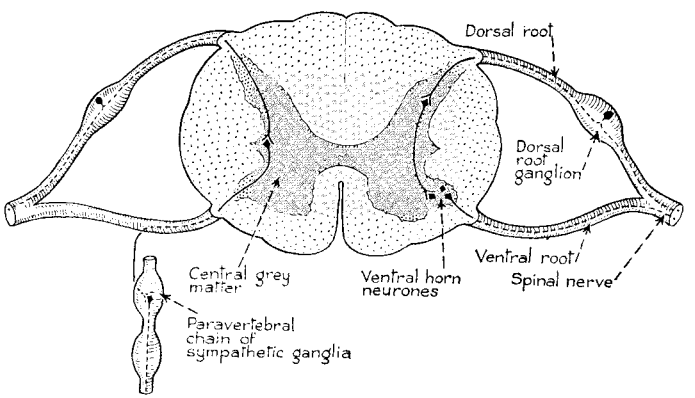


FIG. 12. Cross-section of the spinal cord, passing through nerve roots.

of the cellular core of the spinal cord, the central gray matter. Processes of these motor neurones pass out in the ventral nerve roots, and eventually end on striated muscle fibres. As well as these somatic sensory and motor nerve fibres, spinal nerves also contain fibres which are concerned with the regulation of activity of the blood vessels and viscera, which make up the sympathetic division of the autonomic nervous system. Sensory fibres from the viscera come from nerve cells lying in the dorsal root ganglia, while the visceral motor cells lie outside the central nervous system in a chain of ganglia, linked by strands of nerve fibres, lying on the paravertebral muscles. These cells are brought under the control of the central nervous system by means of axons of

cells lying between the dorsal and ventral groups of cells in the gray core of the spinal cord, called the intermedio-lateral column of cells. Axons of these cells pass out in the ventral roots of the spinal nerves, but leave these by slender bundles of fibres forming rami communicantes, and enter the sympathetic chain. These are pre-ganglionic fibres; they synapse with the neurones of the sympathetic ganglia, some of whose branches, which are post-ganglionic, re-enter the spinal nerves and are distributed with them.

The spinal part of the nervous system thus consists of a series of sensory cells lying in the dorsal root ganglia, and a series of motor cells lying in the ventral part of the central cellular part of the spinal cord. The central processes of the sensory cells enter the cord, and either make contact with second-order sensory cells, or pass to other levels of the nervous system. A complex system of intercalated neurones forms a link between sensory and motor sides of the system, and the actual motor neurones are played upon by fibres linking them with ganglionic neurones lying at the same segmental level, with sensory fibres of sensory nerves at a higher or lower level in the cord, and by descending fibres from higher levels of the nervous system. The pattern of organization of the spinal cord is essentially applicable to the whole vertebrate central nervous system. Input and output divisions can be distinguished, and linking these two, a correlative system of cells and fibres. At higher levels structural and functional complexity has tremendously increased, and the intercalated or correlative structures have grown to form a major part of the system, as in the mammalian cerebellum and in a large part of the mammalian cerebral cortex. In these parts of the nervous system, however, the cellular tissue lies superficially and the white matter, composed of myelinated and unmyelinated nerve fibres, lies deeply, whereas in the spinal cord, the ascending and descending tracts of fibres surround the central gray.

At the microscopic level, the dorsal root ganglia are some of the simplest cellular structures in the nervous system. They consist of a group of neurones, each with a single process arising from

the cell body, which divides into two soon after its origin. One of these branches passes out through the dorsal root on which the ganglion lies, and runs in a peripheral nerve to some receptor structure (see p. 109), which may lie in some viscus, in relation to skin, tendon or muscle, or indeed in any tissue from which afferent impulses are fed into the nervous system. The other process of the neurone passes via the medial part of the dorsal root into the spinal cord, and there, according to the type of stimulus mediated by it, either makes synaptic contact with neurones of the dorsal gray matter near to the point where it enters, or else divides, one branch travelling caudally for a short distance before making contact with neurones, the other passing rostrally for a variable distance before terminating. Nerve fibres within the spinal cord frequently give off collateral branches before they arrive at their destination.

Sensory ganglia such as those of the dorsal roots, and of certain of the cranial nerves, contain neurones which, apart from their particular characteristics such as size, and a specific arrangement of their processes, show the typical features of nerve cells described on p. 46. The cells are usually large, and the Nissl material prominent, although less so than in the large motor neurones lying in the ventral gray matter (Plate VIII, Fig. 30). They differ from the majority of nerve cells in that there is no synaptic contact with the cell body, which simply gives rise to two processes, the peripheral one conducting impulses towards the cell, and the central one away from it. In both cases the direction of conduction is towards the central nervous system. Ganglionic neurones are not the only cellular elements found in the ganglia; the nerve cells are closely surrounded by satellite cells. These are smaller elements which form a kind of cellular covering for the neurones; probably they play a role similar to that of the sheathing glial cells inside the central nervous system. The contents of each ganglion are enclosed within a fibrous capsule.

All neuronal processes found within sensory ganglia of the type described above thus arise from cells actually lying within

the ganglion. This is not the case in other ganglia, such as those of the sympathetic chain, which are motor in function. Ganglia of this type receive preganglionic fibres, via the white rami communicantes, whose cells of origin lie within the gray matter of the spinal cord. These ganglia act as sites where preganglionic impulses are transferred to postganglionic nerve cells, whose processes are widely distributed to the tissues which they innervate. Such ganglia therefore contain synapses; and since a single preganglionic fibre may branch extensively, and make synaptic contact with a number of postganglionic cells, these structures provide a means for the wide dissemination of the preganglionic nervous impulses.

MOTOR NERVE CELLS

The motor nerve cells, which give rise to efferent fibres of the spinal nerves, lie within the central nervous system. In the spinal cord, the motor neurones can be clearly distinguished from the rest of the nervous tissue by their position in the ventral horns of gray matter. This gray matter contains not only the motor cells and their receptor processes, but also the terminal parts of the axons making synaptic contact with them, and the proximal parts of their own axons. The motor neurones differ in structure from those of the sensory ones in the dorsal root ganglia. They are multipolar, having a number of processes extending from the cell; individual neurones are often larger, and their Nissl substance forms coarser granules. As already noted on p. 102, synaptic boutons cover a large proportion of their surface, and these come from a wide variety of other neurones.

Each ventral horn cell gives rise to an axon which passes via a ventral root into a peripheral nerve, and eventually divides to innervate a number of striated muscle fibres. Each single neurone, its axonal process and the muscle fibres which it innervates constitutes a motor unit, and this may include 150 or more striated muscle fibres, all under the control of a single nerve cell. Large motor units such as this are found in the big muscles of the limb girdles; in smaller muscles concerned with more delicate

movements, such as those of the eyeball, the ratio of neurones to muscle fibres is much larger.

The functional complexity of the central nervous system is suggested by its structure. A wide range of neurones in different parts of the nervous system make functional contact with each motor neurone by means of the synaptic boutons, and any motor nerve cell may be under the influence not only of sensory fibres entering the cord at the level at which it lies, but also, either by direct branches or via a system of intercalated neurones, of higher or lower centres of the nervous system.

The presence of an extensive system of intercalated neurones makes it difficult to trace pathways in the nervous system. Lesions of a long fibre tract will of course lead to degeneration along the whole extent of the tract distal to the lesion; but damage to a multisynaptic pathway will leave many of the neuronal links undamaged, so that the pathway cannot be traced by degenerative changes, and recourse must be had to alterations of function and to neurophysiological techniques (see Glees).

THE TRANSMISSION OF IMPULSES

Nervous impulses are electrical in nature; but it is widely believed that transmission of impulses across synapses is brought about by means of chemical substances which are released from the presynaptic axonal process, and which produce a change in the state of polarization of the post-synaptic membrane, which initiates a nervous impulse in the post-synaptic cell.

A number of different chemical transmitters act in this way. One is noradrenaline, and neurones whose impulses are transmitted by release of this substance are called adrenergic. Other neurones, called cholinergic, transmit by release of acetylcholine. In order to limit the effect of this potent transmitter agent to the site where it is liberated, a mechanism exists whereby the liberated acetylcholine is rapidly broken down as soon as it has fulfilled its function of initiating a nervous impulse in the post-synaptic neurone. This destruction is brought about by the enzyme cholinesterase, which hydrolizes acetylcholine. Cholin-

esterase can be demonstrated histochemically; but there is more than one enzyme of this type in nervous tissue, and only specific, or acetyl cholesterinase, is believed to be involved in the breakdown of transmitter acetylcholine. Provided, however, that precautions are taken to distinguish the specific enzyme from others, which are more widely distributed, the histochemical technique can be used to demonstrate sites of cholinergic transmission.

MOTOR END PLATES

One of the most readily studied synaptic sites is in fact a site of transmission of impulses from nervous to non-nervous tissue, namely the point of contact between axonal branches and striated muscle fibres. The pattern of nerve fibres at these end-organs can be studied in histological preparations treated by a metallic impregnation technique, but results are not always satisfactory. These synapses are, however, cholinergic, and their distribution can be strikingly demonstrated by means of the histochemical reaction for cholinesterase. The end plates can be examined in sections, or in whole-mount preparations of teased muscle fibres. In suitable specimens, the branching of the axon of the motor nerve can be followed, each branch ending in a rounded expanded structure on a single muscle fibre.

Although contact between the fine branches of the axon and the muscle fibres is a very intimate one, no actual continuity of axoplasm and sarcoplasm occurs. The fine reticular sheath of the nerve fibre, or endoneurium, becomes continuous with that surrounding the muscle fibre, and terminal arborizations of the nerve fibre lie in grooves in the sarcolemma, without penetrating it; the neurilemmal sheath of the nerve fibres is probably lost at this point. The structure of the end plate has only been accessible to detailed study since the electron microscope was applied to the problem. Electron micrographs show that there is an accumulation of mitochondria in the terminal branches of the axon, as well as in the area of sarcoplasm immediately beneath the end plate; and also that the axons show an accumulation of

the small synaptic vesicles which have been associated with the presence of the transmitter acetyl choline. It is interesting to compare the pictures of motor end plates which result from classical histological and histochemical techniques (Plate VIII, Figs. 31 and 32).

RECEPTORS

The terminal branches of afferent sensory nerves, which are excited by various types of stimuli, may simply lie free in the tissue. More often, however, they are specifically associated with other tissue elements, and form more or less complex structures. A number of different forms of these can be recognized, but only two examples will be considered. The first has already been referred to at an earlier stage; this is the type of branched ending found in the connective tissue lying directly under the endocardial lining of the heart, particularly in the atria around the entries of the great veins. These endings take the form of complex branching nerve fibres, which are arranged so that the greatest extent of the branches is parallel to the endocardial surface. It is extremely probable that these receptors are sensitive to changes of pressure in the atrial cavity, and form the receptors for the afferent side of reflexes controlling the activity of the heart. They do not appear to be associated with any particular arrangement of the surrounding connective tissue, but the fine nerve branches are apparently continuous with irregular swellings, which may play some part in the activity of the organ (Plate IX, Figs. 33 and 34).

The second example considered is one of the class of encapsulated end-organs, in which the termination of the nerve fibres is closely associated with a specialized formation of connective tissue. This forms a Pacinian corpuscle, named after the anatomist who first described it. The structure consists of whorls of flattened cells, forming a rounded or ovoid lamellated body (Plate IX, Fig. 35) measuring up to several hundred μ in diameter. A nerve fibre enters this at one pole, first losing its myelinated sheath, and the neurilemma becomes continuous with the cellular

lamellae of the corpuscle. These organs are found particularly in subcutaneous tissue, in connective tissue in the neighbourhood of joints, and in the mesentery of the intestines. It is believed that they subserve pressure sensation. Recently it has been demonstrated that Pacinian corpuscles may lie on anastomotic channels between arteries and veins, and this finding has given rise to the theory that they may act as indicators for variations in the circulatory pattern of the smaller vessels of the skin (see Cauna, 1962).

NON-NERVOUS ELEMENTS IN THE NERVOUS SYSTEM

The association of nervous elements with non-nervous tissue has been considered in a few special cases, namely the synaptic contact with striated muscle fibres, and the association of nerve fibres with encapsulating elements in certain specialized receptors. Some attention has also been paid to the neurilemmal sheath of nerve fibres, and to the myelin sheath which is often present; but these tissues are so closely associated with the actual axons that it is really necessary to consider the two as forming a functional entity. The same may be said about some of the other elements in the nervous system, namely the neuroglia. These elements, long believed to act as the equivalent of connective tissue in the central nervous system, probably play various roles vital to the continued functioning of the neurones.

Glial elements can be subdivided on developmental, morphological and functional grounds, and three main types are described. The first of these is called micro- or mesoglia, and differs from the other two in that it develops from mesoderm, and migrates into the nervous system with the blood vessels during development. In preparations of normal tissue these cells are relatively inconspicuous; but they are potentially phagocytic, and if tissue of the central nervous system is damaged, they increase in size and ingest the broken-down tissue.

The other two types of glia are both developed from ectoderm. One group, the astrocytes, has an intimate relationship both to capillaries and to nerve cells, and forms a bridge between the

two. Their expanded feet are closely applied on one side to the wall of the vessel, and on the other they come into close association with the surface of a neurone. Such a relationship has suggested that they may be concerned with the transfer of material between the blood and the neurone, or in the reverse direction.

The third type of glial cell is the oligodendrocyte, so called because it has relatively few processes. Cells of this type are found in the tracts of fibres in the central nervous system, and they form a cellular sheath for the fibres in the same way as Schwann cells do in the peripheral nerves. These elements are probably also responsible for laying down the myelin in the central nervous system.

The cytoplasm of glial cells is generally difficult to stain, and as already noted these cells are usually demonstrated by metallic impregnation techniques, although their nuclei are stained by basic dyes. Electron microscopy, however, has enabled us to study the fine structure of glial cells, and has also made possible the disproof of a misinterpretation of histological observations which has held a place in textbooks of neuroanatomy for many years, namely the precise relationship of the finer intrinsic blood vessels of the nervous system to the neural tissue.

The large vessels supplying the brain and spinal cord lie in the subarachnoid space, which lies between the middle and inner layers of membranes covering the central nervous system, that is between the arachnoid and the pia; to the outside of the arachnoid is the thick fibrous dura mater; the arachnoid is a more delicate membrane made up of collagen fibres, and joined to the pia, which is closely applied to the surface of the brain, by delicate trabeculae of collagen. The whole subarachnoid space is lined by flattened mesothelial cells, and filled with cerebrospinal fluid.

Branches of the vessels lying in the subarachnoid space pass deeply into the underlying nervous tissue, and are accompanied in the first part of their course by a continuation of the subarachnoid space, lined by pial mesothelium. It was formerly thought that even the finer vascular branches and capillaries were surrounded by a continuation of this space, and that it eventually

communicated with a perineuronal space around individual nerve cells. It is now known, however, that the space accompanies only the larger vessels, and that there is no true space surrounding either the capillaries or the neurones. The idea of a continuous perivascular space arose partly because during fixation shrinkage of the tissues produces spaces where none exist in the living tissue, and partly because of the nature of the glial cells, astrocytes, surrounding the smaller blood vessels.

It has already been noted that end feet of astrocytes are closely applied to blood vessels in the central nervous system, and these end feet may be so numerous that they form a cuff around the vessel. Electron microscopic studies have shown that the cytoplasm of these cells is extremely "watery", and that they contain very little electron dense material. By contrast, neurones and their processes, and other kinds of glial cells, are much denser. Lack of dense material also means lack of stainable material, and hence the watery cytoplasm of the astrocytes added to the illusion that vessels and neurones were surrounded by a continuous perivascular-perineuronal space. In fact, the perivascular space in the central nervous system is so small that cells and processes are virtually in contact with capillaries.

BLOOD VESSELS OF THE NERVOUS SYSTEM

Nervous tissue, and particularly the neurones, are extremely susceptible to any lack of oxygen. Hence the supply of blood, and the structure of the vessels supplying it, to the nervous system is of especial importance. This does not obtain in the simpler nervous systems, such as those of invertebrates, in which the tissue can be supplied with its metabolic requirements by a process of simple diffusion from the surface. Similarly, in higher forms of life, the fine terminal filaments of the peripheral nerves require no special vascular organization, since they can be supplied by the same processes as any other intervascular tissue. Where nervous tissue forms any appreciable mass, however, the vascular system becomes specialized in some way.

Thus even the peripheral nerves, in all parts except their fine

terminal branches, have their own system of blood vessels in the form of longitudinal anastomotic channels running along the nerves; in the case of the larger nerves, quite large arteries, which can be dissected out without using a microscope, run to the nerves to supply these channels. These vessels run in the connective tissue which forms the interstitial tissue of the nerves.

The main mass of the central nervous system has a much more complex vascular system, which corresponds to one of two main patterns, according to the species. Both have main arterial and venous vessels, supplying and draining away the blood, which lie superficially; but it is the intraneural pattern of finer vessels which is characteristic of one or the other type (Fig. 13).

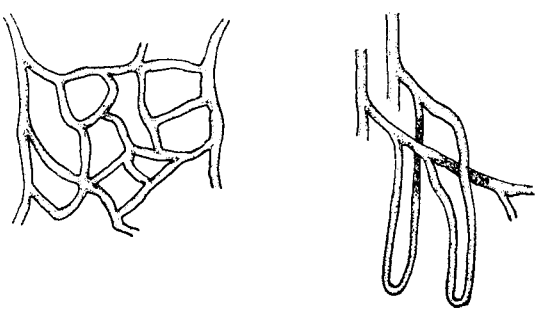


FIG. 13. Net (left) and loop patterns of blood vessels in the central nervous system.

The more primitive, which is found in some invertebrates, in vertebrates such as reptiles and also in a mammal, the opossum, consists of closed capillary loops uniting the arterial and venous sides. Each loop is responsible for the supply of a small territory of nervous tissue, of the order of 25 μ radius, which is limited by the distance over which effective diffusion can occur. The other type of system, which is found in the majority of mammals including man, consists of a network of capillaries joining the arterial and venous sides of the circulation, and in such a system all cerebral and spinal vessels are interconnected by a capillary bed.

Theoretically, blockage of one of the vessels feeding into the capillary network of the latter type of vascular system might be expected to have no serious effect on the nervous tissue, since the field of supply of the blocked vessel could be taken over by vessels lying to either side of it. In practice, however, it is found that blockage of a supplying vessel is followed by death of a greater or lesser zone of nervous tissue, depending on the size of the affected vessel, so that despite structural continuity of the vascular bed, functional continuity is not adequate.

The pattern of the vessels varies greatly in different parts of the nervous system. Areas which are densely cellular and contain many large neurones usually have a denser capillary network than less cellular regions, or than tracts of fibres. The density of the capillary bed may, however, depend not so much on the numbers of cells present, but rather on the numbers of synaptic contacts on these cells. The ganglion of the fifth nerve, for example, contains numerous nerve cells, but no synapses, and does not have a dense capillary bed.

Vessels of the nervous system contain enzymes in their wall which can be demonstrated by appropriate histochemical techniques. Capillaries usually have a high content of alkaline phosphatase, and as previously mentioned, this enzyme may be associated with the transfer of material across the wall. In some species, such as the rat and the rabbit, capillaries of the central nervous system have a high content of cholinesterase. In the rat this is of the non-specific type, and may not be particularly associated with the hydrolysis of acetylcholine, or indeed of other cholinesterases; but in the rabbit, capillaries in certain nuclear areas or tracts of the brain stem contain specific cholinesterase; this is an interesting observation, but like so many other histochemical data, its significance is so far not understood.

EXCRETORY TISSUE

The intestinal tract, lungs and kidneys can all be considered to play a major part in the elimination of waste products from the body; but of these, only the kidneys are concerned predominantly

with excretion; the intestine is mainly involved in absorption, and serves as an excretory tissue chiefly by acting as a channel for removal of the biliary secretion from the body; while the lungs are concerned with the oxygenation of the blood as much as with elimination of its carbon dioxide.

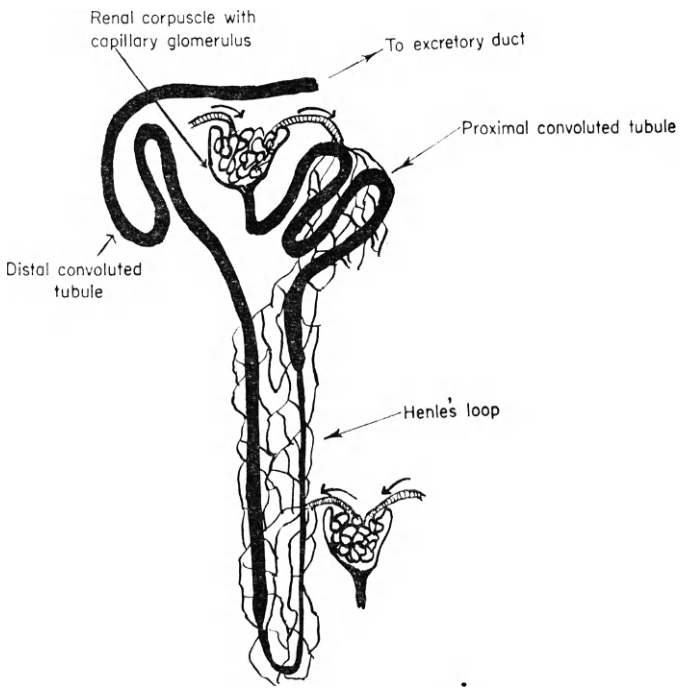


FIG. 14. A single nephron and its associated blood vessels. The cortex of the kidney would lie at the top of the diagram, and the medulla towards the bottom. A juxta-medullary glomerulus is shown to the lower right of Henle's loop, and the drainage of its efferent arteriole into capillaries around the loop indicated. (↑) direction of blood flow.

The kidneys are primarily concerned with the elimination of the non-gaseous break-down products of tissue metabolism from the blood, and since these waste products from the various tissues

enter the blood flowing through the various organs, the kidneys are indirectly the excretory organs for them all. Essentially the kidneys are made up of large numbers of tubules, intimately related to blood vessels, into which dissolved waste products are passed; since however an excess of water, and certain other substances which are required by the body, also pass into the tubules, provision is made for the selective reabsorption of certain constituents of the initial filtrate; and the final product containing waste products is excreted as urine.

The characteristic structural and functional unit of the kidneys is a vascular-tubular complex called a nephron. Each human kidney is made up of over a million of these. A single nephron consists of a long tubule, which originates in an expanded blind end, Bowmans capsule, which is invaginated by a leash of capillaries called a glomerulus, arising from a single afferent arteriole and draining into a single efferent arteriole. These capillaries are closely invested by the epithelium of the blind end of the tubules (Fig. 14), and it is across this barrier, formed by the wall of the capillary and the single investing layer of tubular epithelium, that water and dissolved substances are passed, to enter the lumen.

The passage of this water and its dissolved substances across the barrier is probably a process of simple filtration, and larger molecules, such as those of the plasma proteins, are prevented from crossing, provided that the barrier is not damaged; in certain pathological conditions, however, such substances may cross and be excreted in the urine. Unlike some other parts of the nephron described below the glomerular complexes have no marked content of alkaline phosphatase, and as already noted, this enzyme is often associated with the active transfer of substances such as glucose across membranes.

Bowman's capsule leads in to the next division of the nephron, which is called the proximal convoluted tubule. This is a tortuous part, which lies near the glomerulus; it is formed by a single layer of pyramidal cells which have a conspicuous brush border. The proximal convoluted tubule leads into a U-shaped loop which

passes towards the hilum of the kidney, then turns and passes back towards the vicinity of the original glomerulus, where it becomes continuous with the distal convoluted tubule. The U-shaped structure is known as Henle's loop; its first or descending part is lined by flattened cells with little cytoplasm; the second or ascending part of the loop is thicker, and lined by low cuboidal cells. The distal convoluted tubule is lined with cells which are more flattened than those of the proximal one, and have no brush border. These tubules are linked to a system of excretory ducts which pass to the hilum of the kidney, and open into the upper expanded part of the ureter which conducts the urine to the bladder.

The kidney can be divided into a cortex and a medulla. The renal corpuscles lie in the cortex, together with the proximal and distal convoluted tubules. The loops of Henle pass down into the medulla; while the collecting tubules, which join with the terminal part of the distal convoluted tubule, extend from the cortex down through the medulla, becoming larger as the hilum is approached and more and more small collecting ducts open into the main ones.

The arrangement of the blood vessels of the kidney closely follows that of the tubules, and this correspondence emphasizes the functional interdependence of these two components, forming a single physiological unit. The pattern of the vessels differs to some extent from that of the tubules, however, in that the efferent arterioles from glomeruli lying in the outer cortex of the kidney supply capillary beds surrounding the proximal and distal convoluted tubules. Capillaries of the inner cortical glomeruli, however, contribute blood both to capillary beds in the inner cortex, and also to straight vessels which pass down into the medulla, and lie in close association with the loops of Henle.

The function of the renal corpuscles has already been dealt with. The result of the selective filtration of water and certain constituents of the plasma across the membrane formed by the glomerular endothelium and the closely applied capsular cells is

that the fluid within the first part of the nephron is both more dilute than urine, and also contains substances such as glucose which are not normally found in urine. A major role played by the rest of the nephron is the selective reabsorption of those substances present in the initial filtrate whose loss would be detrimental to the animal. This reabsorption is in some respects at any rate an active process on the part of the tubular cells, although as far as reabsorption of water is concerned, osmosis seems to play a major role. But although reabsorption is a dominant activity of the tubules, it is not the only one, since tubular cells have also some excretory capacity, and substances can be passed from the blood into the tubular lumen.

Absorption of different materials occurs in different parts of the nephron. The high content of alkaline phosphatase in the brush border of the cells lining the proximal convoluted tubule is probably associated with the active transfer of glucose across the wall of the tubule back into the blood. Provided the concentration of glucose in the blood is below a certain level (180 mg per cent for the human) all that originally present in the glomerular filtrate is usually absorbed, and none remains in the urine. Sodium chloride and phosphates are also absorbed by this proximal part of the nephron, together with an appreciable part of the water. It is an important point, so far as this latter activity is concerned, that the blood in the capillary bed around the proximal convoluted tubules has already passed through the glomeruli, and hence has become concentrated by the loss of water, while retaining its content of protein; hence there is an osmotic gradient from tubular fluid to the capillary blood.

The anatomical arrangement of tubules and vessels in the kidney is difficult to appreciate from thin sections, although these reveal the intimate relationship of the two elements. Thick sections of injected specimens give a better idea of the pattern of blood vessels, particularly those in relation to the loops of Henle which descend into the medulla and then return again into the cortex. The arrangement in this part of the kidney forms the structural basis of the "hairpin countercurrent mechanism"

which is believed to play a major role in the process of reabsorption which occurs in renal tissue.

The evidence suggesting that the countercurrent mechanism may apply in the functioning of the kidney comes from experimental analyses of the fluid contained in the nephron at various levels, and on estimations of the concentration of solutes in the renal parenchyma in samples taken from the outer cortex to the inner medulla. The findings of such studies can be briefly stated as follows:

(a) Active absorption of glucose and sodium and other ions in the upper part of the nephron is accompanied by the absorption of water. The result is that the descending limb of Henle's loop contains a fluid which is hypertonic to the blood plasma.

(b) Sodium is actively absorbed from the ascending (thick) segment of Henle's loop, and passed initially into the interstitium of the kidney.

(c) The increased concentration of sodium in the interstitium which results from (b) brings about the extraction of water from the descending limb of Henle's loop, on account of the osmotic gradient created by the increased ionic concentration in the interstitial fluid surrounding this latter structure.

Since fluid is lost increasingly from the descending limb as it passes towards the medulla, and sodium increasingly from the ascending limb as it returns towards the cortex, the maximum tonicity of intratubular fluid is reached at the bend of the loop, where the descending limb turns back into the ascending one. The result of this mechanism is that a hypotonic fluid is delivered from Henle's loop into the distal part of the nephron, that is the distal convoluted tubule, which lies in the region of the glomerulus supplying the nephron in question. The water and sodium which leave Henle's loop and enter the interstitium of the medulla, are absorbed by the capillary blood vessels of that part of the kidney which arise from the deeper cortical glomeruli. Since the glomerular filtrate has already been removed from the blood in these vessels, they also contain a relatively hypertonic plasma.

Thus by means of a somewhat complex mechanism, hypotonic

fluid enters the distal part of the nephron; and it is this part of the tubular system which appears to be largely under the control of antidiuretic hormone secreted by the neurohypophysis. This hormone brings about the absorption of water from the distal convoluted tubule, and if there is a deficiency of hormone, the urine excreted by the organism will be more dilute than usual, have a lower specific gravity, and also be greater in volume. Other hormones, such as those of the adrenal cortex and the thyroid, also influence the concentration of the urine, so that tubular activity is not under the sole control of the posterior pituitary, but also influenced, probably indirectly, by adeno-hypophysial secretions.

The remaining part of the urinary system is usually thought of only as a system of ducts for the conduction of the urine from the distal part of the nephron, its storage in the bladder, and its eventual discharge. There is evidence, however, that at any rate the proximal part of the collecting ducts can also absorb fluid to some extent, probably under the influence of the antidiuretic hormone. The lining of the small collecting ducts, which are continuous with the distal convoluted tubules, consists of a single layer of low epithelial cells, which however become tall columnar in the larger intra-renal ducts. The main duct from each kidney, the ureter, is lined by an epithelium several cells in thickness, whose appearance changes according to the degree of distention of the tube. This epithelium rests on a basement membrane, outside which is a longitudinal layer of smooth muscle, surrounded by a circular layer. Peristaltic contractions of these muscular layers assist the passage of urine to the bladder where it is stored until voided. The bladder is a distensible muscular organ for the storage and forcible expulsion of urine; like the ureter, it is lined by several layers of epithelium with no secretory or absorptive properties.

RESPIRATORY TISSUE

The lungs consist of two main parts, namely a system of ducts carrying the respiratory gases, and the respiratory tissue itself

where the actual gaseous exchange between air and blood occurs. The duct system originates in the trachea, which divides into the main or primary bronchi, one of which passes to each lung. At the root of each lung the main bronchi become associated with the arteries, veins and lymphatics of the lungs, and each primary bronchus gives a branch to a lobe of the lung. This large lobar bronchus passes out into the tissue of its respective lobe, progressively dividing into smaller and smaller branches until tubes of less than about 1 mm in diameter are formed. These, called the bronchioles, are the terminal part of the duct system, and lead to the respiratory part of the lung.

The trachea in the human is in cross-section a U-shaped tube lined by a single layer of columnar epithelium, which appears to be pseudo-stratified, since nuclei of the epithelial cells do not all lie at the same level. These cells are ciliated, and the cilia beat towards the pharynx. The epithelium also contains goblet cells, which secrete mucus. As commonly found with epithelia, it rests on a lamina propria of connective tissue, and a well marked basement membrane lies directly beneath the epithelium. The lamina propria contains numerous elastic fibres, scattered lymphocytes, and occasional lymphoid nodules.

Outside the lamina propria lies the submucosal layer, which contains small mucous glands, whose secretions pass into the lumen of the trachea via small ducts. The wall of the trachea is strengthened by a series of incomplete rings of cartilage, deficient posteriorly, each separated from the ones above and below by dense connective tissue. The posterior gap in the cartilage rings contains, in addition to connective tissue, interlacing bundles of smooth muscle fibres.

The smaller ducts of the respiratory system, lying within the lungs, have the same general structure as the trachea. The walls are of course thinner, and the lining cells tend to be lower, that is, cuboidal rather than columnar. The cartilage rings are replaced by irregularly shaped plates, and the smooth muscle now forms an incomplete coat all round the bronchioles, inside the plates of cartilage. Contraction of these muscle fibres may considerably

impede the flow of gases through the smaller bronchi. By the time that the bronchioles are reached there is no cartilage in the walls, and the epithelium contains neither goblet cells nor cilia in the smaller branches. A muscular coat is present, and the walls are attached to the elastic network that forms a framework for the surrounding respiratory tissue.

The terminal parts of the bronchial tree are small respiratory bronchioles which branch and lead into alveolar ducts, off which open the alveoli (Fig. 15). These are virtually small cellular

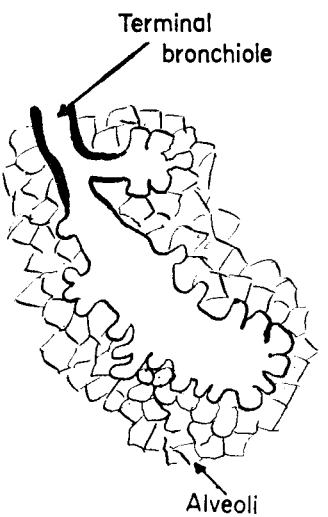


FIG. 15. The terminal part of the duct system of the lungs. A respiratory bronchiole lies surrounded by alveoli.

chambers separated from each other by common walls, and it is here that the respiratory exchange takes place. The alveoli illustrate two points; firstly, a high degree of structural specialization for a particular function; and secondly, how difficult it is to interpret the finer details of the organization of certain tissues without the aid of the electron microscope.

The inter-alveolar walls are extremely thin in relation to the

size of the alveoli themselves, and are best studied, using the light microscope, in thick sections which will cut through two adjacent alveoli, and contain the whole thickness of the wall separating them. A further point which must be considered is the method of fixation of lung tissue; as soon as the chest is opened the lungs collapse, due to the shortening of the elastic meshwork described below, which is normally stretched by the negative pleural pressure which keeps the lungs expanded to fill the cavity of the thorax. The lungs are therefore best fixed by injection of the fixative via the trachea, so that they are distended to the size they were during life.

If an inter-alveolar wall is examined in a thick section it can be seen to contain large numbers of nuclei, but it is often difficult to make out the precise structural details of the wall. In transverse section this is extremely thin, provided the lungs were not fixed in a collapsed state, and contains prominent thin-walled capillaries. Thick sections of material in which the blood vessels have been injected with indian ink or some other medium, however, show that the meshwork of capillaries in the interalveolar septa is extremely dense, and forms a major component of the wall. These delicate vessels are supported by basement membranes and by delicate reticular or collagenous fibres. Overall support for the respiratory tissue comes from a rather widely spaced mesh of coarser elastic fibres, but these give little support to individual capillaries.

Most of the nuclei seen in the alveolar walls belong to the endothelial cells of the capillaries; but an appreciable number belong to septal cells, which lie within or on the walls. These cells are potentially phagocytic, and will ingest foreign material, which may in certain pathological conditions consist of blood which has extravasated into the alveoli. These cells then move along the air passages to the bronchioles, and eventually are coughed up.

For many years there was uncertainty about the precise nature of the lining of the alveoli, and it was debated whether or not there was any continuous cytoplasmic lining separating the

lumen of the alveoli from the capillary walls. Studies using the electron microscope have now shown that there is a continuous lining of cytoplasm, extending out from the perinuclear region of the septal cells, but that this cytoplasm is only of the order of a quarter of a micron or less in thickness. Hence it is scarcely surprising that studies using the optical microscope failed to resolve this problem with any certainty. The endothelial cytoplasm of the capillary wall is of the same order of thickness; and since, except where a tissue space intervenes between the septal and endothelial cells, the two elements are separated only by their respective basement membranes, it can be seen that there is only a very slender barrier between the alveolar air and the blood. It is of course across this barrier that respiratory exchange takes place.

CHAPTER IV

The differentiation of cells and tissues

The final section of this book is a brief consideration of the differentiation of cells and tissues. Although this topic properly belongs to embryology, we cannot afford to ignore the process of differentiation even though mature tissues are the main object of study. The way in which a tissue is formed often gives considerable information about the structural and functional organization of the mature tissue; and modern techniques, such as histochemistry, applied to developing tissues, may give valuable data on the onset of activity.

Animals develop from the fertilized egg, a simple cell whose nucleus has regained a full (diploid) complement of chromosomes by the combination of the male and female pronuclei, which as a result of meiotic division contain only a half (haploid) complement of chromosomes each. This single fertilized egg cell must have the potentiality of forming all the cells of the mature organism.

Studies of the development of an organism from the one-cell stage may be concentrated either on the development of individual cells, cytogenesis; on the formation of tissues, histogenesis; or on the formation of organs, organogenesis. During the earliest stages of the formation of any tissues the cells are largely undifferentiated from each other as far as their appearance under the microscope shows, and it is often impossible to distinguish, on cytological grounds alone, between cells destined to form

different tissues. For example, many organs develop as epithelial outgrowths from the primitive gut. The lungs arise from the primitive oesophagus, and the liver and pancreas are formed as outgrowths from the mid-gut. In the earliest stages of development, however, the epithelium lining the primitive viscera consists of one or several layers of cells which generally resemble each other so closely that it is impossible to distinguish those destined to give rise to one or other of these organs, except by taking into account the position and form of the outgrowths. Even after the essential structure of the organs has become established, many of the cells may still appear relatively undifferentiated. On the other hand, some tissues acquire distinctive morphological features relatively early, and their constituent cells can be recognized individually as belonging to a certain type, even without reference to their situation in the embryo. Thus the primitive nerve cells, or neuroblasts, forming ganglia can be recognized as such by their cytological features early in the course of development.

Rapid proliferation is a characteristic feature of embryonic tissues, and they therefore have a high level of synthetic activity, particularly for protein. The intensity of the growth processes is illustrated by the case of the embryonic chick, which increases in weight about twenty-fold between the second and tenth days of incubation. The cytological features associated with such a rapid rate of cellular proliferation are similar to those already considered as demonstrating a high rate of synthetic activity in mature cells (see p. 44), namely large nucleoli, and a high concentration of cytoplasmic RNA which appears in sections stained by many histological techniques as an intense cytoplasmic basophilia. Such features are also found in growing non-foetal tissues, as well as in those undergoing regeneration.

A characteristic of histogenesis however is differentiation, and as this occurs the cells gradually acquire the characteristics of mature cells. Thus developing muscle cells form myofibrillae in their cytoplasm and assume their characteristic elongated form. Exocrine glandular cells become orientated with respect to the

lumen into which their secretion will be passed, and their nuclei come to lie basally in the cells, away from the lumen and nearer to the underlying vascular stroma; at the same time the cells acquire staining reactions resembling more closely those of mature tissue. Not all cells of a tissue, however, differentiate in this way at the same time, and a certain number retain their primitive characteristics, and by dividing mitotically give rise to more cells of the tissues concerned.

As already noted, the recognition of cells as being of a type characteristic of any particular tissue may not be easy during the developmental phase, unless the location of these cells and their relationship to each other is also taken into account. The problem is more difficult in cases where cells destined to form an organ or tissue do not proliferate *in situ*, but migrate from some other situation in the organism. In some cases, it may still be relatively easy to determine the source of cells. A case in point is the developing adrenal gland, whose medullary cells are derived from the neural crest, and migrate ventrally to enter among the mass of cells which give rise to the cortical part of the gland, derived from the lining of the dorsal wall of the coelomic cavity. The migrating cells can be readily seen in sections through the embryo, extending along a line from the neural tube to the site of the developing adrenal, and by studying embryos in various stages of development it is relatively easy to trace the progress of these cells towards their destination. The problem is more difficult in cases where cells migrate before they are sufficiently differentiated to be recognizable in stained sections, or where they are indistinguishable from the tissue through which they are migrating. The germ cells provide an example of this type. The primitive cells which eventually give rise to the ova in female and spermatozoa in male animals, are believed to originate in the dorsal mesentery of the gut, and at an early stage of foetal development migrate to the developing gonads. Histologically, however, it is impossible to distinguish these cells at the stage when they are in the mesentery; but fortunately they have been found to contain a high concentration of alkaline phosphat-

ase, and since the cells amongst which they are lying do not, it has been possible to apply the histochemical technique for this enzyme to the demonstration of germ cells. Again, a study of a progressive series of developing embryos enables the passage of these elements to be traced, still by virtue of their content of alkaline phosphatase, as they migrate from their original site into the developing gonads.

The appearance of characteristic morphological features in developing cells provides clear evidence of cellular differentiation. In the human foetus, for example, the epidermal part of the skin does not develop its characteristic appearance until the fourth month of development, and at this time keratin appears as a component of the more superficial cells. The development of structural features associated with cellular maturity need not however indicate the actual onset of some specific activity; and on the other hand, functional activity might occur before those features usually associated with it in the mature cell have appeared. According to Goss, the rudiment of the heart in the embryonic rat begins to contract on the tenth day of development, and this contractile activity occurs some hours before the myofibrils and cross striations typical of myocardial cells have appeared. These contractions occur at a stage when the heart is extremely undeveloped as an organ, and before nerve fibres have grown into the myocardial tissue. Contractility thus appears to be an intrinsic property of myocardial cells, not necessarily associated with their cross-striated structure.

Histochemical methods may give some indication of the onset of function in developing tissues. In the case of secretory cells the accumulation of secretory products in the cytoplasm is an indication that at any rate the synthetic part of the secretory process is taking place, although liberation of the synthesized products may not be occurring. Mature liver cells store glycogen; and the appearance of glycogen in developing liver cells is therefore indicative that they are beginning to function. In the chick glycogen can be demonstrated, by the use of the PAS technique, on the seventh day of incubation; the secretion of bile, which is

another function of liver cells, appears however a day earlier than this.

A further example of this type is afforded by the foetal pituitary. The adenohypophysis consists at an early stage of its development of cords of basophilic cells which are arranged round lumina derived from the cavity of Rathke's pouch, which grows up from the buccal epithelium from which the gland develops. The whole structure appears in sections to be made up of rosettes of cells, separated after a certain stage in development by loose connective tissue containing cells and carrying blood vessels into the gland. The cells which will form the secretory elements of the gland enlarge as they differentiate, and eventually assume characteristic staining reactions. As already discussed (p. 80) some of the hormones secreted by the adenohypophysis are glycoprotein in nature, and can therefore be selectively demonstrated by the PAS reaction. Jost, in studies of the developing endocrine system of the rat, noted that a group of PAS-positive cells appeared in the gland for the first time at the 15th day of gestation, and that another group appeared at 17 days. He noted that the interstitial cells of the testes were well developed at 16 days, and that the thyroid showed first signs of activity at 18 days, and suggested that the maturation of these two components of the endocrine system, which are known to be dependent on the adenohypophysis, could be correlated with the beginning of secretion of gonadotrophic and thyrotrophic hormones by the latter. Furthermore this secretion could apparently be correlated with the appearance of PAS-positive material in the two groups of PAS-positive cells appearing in the adenohypophysis at the 15th and 17th–18th days respectively. Further data resulted from Jost's experiments using rabbit embryos, in which he observed that from the 21st–23rd days of gestation a number of cells containing large strongly PAS-positive granules developed, and that by the 24th day all such large inclusions had disappeared. Jost correlated the discharge of these granules with the development of the genital tract, since he found that hypophysectomy of the foetus by decapitation before discharge of the granules was

followed by retardation in the development of the genital tract. Decapitation at a later stage of development, after the cells had discharged their granules, was not followed by any retardation of development of the tract. Jost here applied a common method of experimental embryology, namely the removal of an endocrine organ at different phases of foetal development, to see whether removal is associated with some evidence of deficiency in the target organs. Unfortunately the minute foetal pituitary gland cannot yet be removed surgically, except by the somewhat gross procedure of decapitation, or X-irradiation.

Just as morphological features are not necessarily correlated precisely with activity, so the appearance of histochemical features usually associated with some specific function does not necessarily indicate that the cells have already begun to function in that way. For example, the pigment melanin which is present in epidermal cells of the skin is formed by melanoblasts (see p. 12) which can be distinguished from cells which contain pigment, but which do not manufacture it. Melanoblasts produce pigment during foetal life in dark-skinned races, whose children are born with pigmented skin. Little pigment is, however, present at birth in the skin of foetuses of white races, since little or none is produced during foetal life. Nevertheless melanoblasts of white foetuses show a characteristic histochemical reaction, typically associated with formation of pigment (Dopa reaction) some months before birth. This histochemical feature suggests that enzymes capable of manufacturing pigment are already present in the cells at this time, although they are not yet functional.

In the case of many tissues there is no ready way of demonstrating the specific products of cellular activity. For example, the thyroid gland consists of epithelial cells arranged to form follicles, and these follicles contain amorphous colloid which is rich in thyroid hormone. There is no simple staining reaction which can demonstrate the amount of hormone present in the colloid, so that the onset of activity of the gland has to be shown by more complex methods. One method is to extract the active principles from the gland, and correlate the histological maturation with the

appearance of biological activity. Alternatively, a method can be used which involves the uptake of some substance which is known to be incorporated into the secretory material. This type of technique is particularly appropriate in the case of the thyroid gland, since thyroid hormone contains a large amount of iodine in its molecule. The thyroid follicular cells concentrate iodine from the blood and incorporate it into the hormone. The onset of synthetic activity in the thyroid can therefore be shown by injecting radioactive iodine into the foetus, and determining at what stage this is first taken up by the thyroid cells, and when it first appears in the colloid. Such studies may make use of autoradiographic techniques, or direct counts of the number of β-particles emitted by the thyroid tissue can be made, and hence the amount of radioactive material taken up be determined. Experiments of this nature have shown that in the rat the onset of activity in the cells of the thyroid occurs at about the 18th or 19th days of gestation, that is at about the same time as morphological differentiation of the follicles occurs.

Conclusion

A REASONABLY complete understanding of structural and functional correlations in animal tissues can only be gained by making use of the most appropriate techniques available for studying any given tissue, remembering that no single approach can be expected to give a full appreciation of either structure or function. Histological studies using the light microscope still occupy a major place in biological investigations. The main limitations of histology have already been described, and it is clear that any histological preparation depicts the state of a tissue at one instant only, that of its fixation; furthermore, the tissue is examined in an artificial state, after treatment with fixatives, dehydrating, embedding, sectioning and staining. Artifacts may be produced at any of these stages of preparation, and the observer must learn to recognize these. Such artifacts become even more important when the electron microscope is used, on account of its great resolving power and high magnification.

Despite the drawbacks, histological studies, whether carried out using the optical or the electron microscope, can be of the greatest value in enabling a picture of the activity of tissues to be constructed. In the first place, structural studies reveal details of the organization of tissues, and these reflect their activity. The relationship of the renal tubules to blood vessels, and the rich vascularity of the endocrine glands, are cases in point. Structural studies alone can also give direct indications of activity. Specimens of tissues can be collected at different phases of a cycle of activity, and the morphological basis of the cycle determined. Quantitative methods can be applied; and now histochemical techniques, essentially an extension of histology, can be used to gain an idea of chemical processes going on in the tissues.

The newer techniques must be founded on a base of structural studies. Phase-contrast and other methods of microscopy extend the limits of the optical microscope; radioisotopes can be used to reveal dynamic processes going on in cells; and cultures of tissues can be studied while their components are living, although necessarily in a somewhat artificial environment. In a brief text such as this it has been possible only to select a few examples of the way in which a multilateral approach can give a reasonably comprehensive picture of the structure and function of tissues. Fuller descriptions of the organization of animal tissues, methods of studying their functions, and the results of such studies, will be found in the selected textbooks, monographs and articles which follows.

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Appendix

A FEW USEFUL STAINING TECHNIQUES

1. HAEMATOXYLIN AND EOSIN: A GENERAL STAIN

Wax sections. Bring to water through xylene and graded alcohols.
Stain in ripe 0·5 per cent aqueous haematoxylin 15 min.
Rinse in water, then in 1 per cent hydrochloric acid in 70 per cent ethyl alcohol until only nuclei retain the stain.
Wash in running water until the blue colour of the dye is restored.
Stain in 1 per cent aqueous eosin, 5 min.
Wash, dehydrate, clear in xylene and mount in Canada balsam.

2. THE PERIODIC ACID-SCHIFF (PAS) REACTION

The schedule given here is a simplified one, suitable only when the PAS reaction is being used to give a general histological stain; if used as a critical histochemical technique, more care must be taken (see Pearse, 1960).
Bring sections to water.
Treat with an aqueous solution of 1 per cent periodic acid, 5 min.
Wash. Place in Schiff's reagent for 20 min.
Wash for about 10 min. or until the sections are well coloured.
Stain nuclei, if desired, with haematoxylin; sections may be counterstained after this. An aqueous solution of 1 per cent Orange G and 2 per cent phosphotungstic acid is a suitable counterstain for anterior pituitary tissue; in this case stain for only 15–20 sec, rinse and dehydrate rapidly, then mount.

3. ALCIAN BLUE: A STAIN FOR NEUROSECRETORY MATERIAL

Bring sections to water.
Oxidize for 1–2 min in a fresh solution of 2·5 per cent potassium permanganate, 10 ml, and 5 per cent sulphuric acid, 10 ml

diluted to 60 ml with water. Rinse and remove the brown colour with a weak solution of potassium metabisulphite. Wash.

Stain in an aqueous solution of 3 per cent Alcian blue in 2 N sulphuric acid, for 15–30 min. (The dye must be dissolved by heating to about 70°C, and the solution filtered when cool.) Wash. Sections can then be treated by the PAS technique, and counter-stained as desired. NSM stains deep blue.

4. STAIN FOR LIPID; OIL RED 0

Use frozen sections of material fixed in formol-saline, mounted on slides and allowed to dry thoroughly.

Stain in a solution of Oil red 0 for about 10 min; wash, and mount in glycerine jelly. Lipids stain red.

(Oil red 0. Stock solution consists of a 0·5 per cent solution of the dye in isopropanol. For use, dilute with an equal part of water, allow to stand for several hours and filter.)

5. NERVE FIBRES IN PARAFFIN SECTIONS. SILVER METHOD OF W. HOLMES

Bring sections to water. Immerse in 20 per cent silver nitrate 1–2 hr (solution can be used repeatedly).

Wash 10 min in three changes of distilled water.

Place in impregnating solution at 37°C for 24 hr. This consists of 55 ml of boric acid buffer (12·4 g boric acid in 1000 ml distilled water), 45 ml borax buffer (19·0 g borax in 1000 ml distilled water); mix in a 500 ml measuring cylinder, and dilute to 494 ml with distilled water. Add 1·0 ml 1 per cent aqueous silver nitrate and 5 ml 10 per cent aqueous pure pyridine.

After impregnation, reduce for not less than 2 min in a solution of 1 g hydroquinone, 10 g sodium sulphite and 100 ml water. Wash for 3 min; rinse in distilled water. Tone in 2 per cent gold chloride 3 min. Rinse, and treat with 2 per cent aqueous oxalic acid for 3–10 min until axons are black. Rinse, treat with 5 per cent sodium thiosulphate 5 min; wash, dehydrate and mount.

Details of many other staining methods and practical instructions for general histology, can be found in *Handbook of histopathological techniques*, by C. F. A. Culling: Second edition, 1963, London, Butterworths. Histochemical techniques are well dealt with in Pearse's book (*see* References).

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Numbers refer only to the *principal* pages on which the subjects are discussed. Italic type indicates figures on Plates I–IX.

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J. COHEN

Lecturer in Embryology, University of Birmingham

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