THE MICROSCOPIC ANATOMY
OF VERTEBRATES

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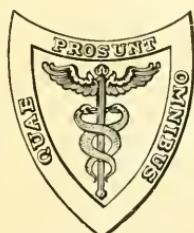
PREFACE.

COURSES dealing with the anatomy of vertebrate animals form an important part of the curriculum of the college program of studies. Work in anatomy is almost wholly occupied with the gross anatomy revealed by dissection of representative vertebrates. Associated with the course in anatomy is work in embryology in which the emphasis is usually placed upon development in the chick and pig. In addition to embryology and gross anatomy, the student is well repaid by continuing his anatomical studies and investigating the tissue organization of the various systems.

Although college textbooks in comparative anatomy and embryology of vertebrates are available, most textbooks in histology and microscopic anatomy have been prepared for medical students and the emphasis has been placed upon human material. In the present book, material has been taken from a variety of vertebrates so that it may serve as a survey of the microscopic anatomy of the vertebrates.

It is a college textbook, the purpose of which is to present a working knowledge of vertebrate histology and microscopic anatomy to be covered in a semester period. It is not intended as a compendium of information on all aspects of so wide a field, and one so rich in interest for investigation. Therefore, the book has been kept primarily on a descriptive basis to accompany laboratory work. The possibility of wide variation and exceptions in different species make it unavoidable that students will find discrepancies between certain general descriptions and the specific example they may be studying. Since this is true, it is hoped that the student will be interested in discovering variations and will not be content with mere verification of the text. Believing that it is well for the student to acquire the habit of consulting original papers, the authors have appended a few selected references to each chapter. This list of references is in no way complete, but has been selected from the more readily available publications to serve as a starting-point.

The degree of magnification of the drawings and photographs has been deliberately omitted. The statement to the effect that a certain illustration is 600 or 1000 times the actual size conveys very little helpful information. The student will be constantly using the microscope and will have little difficulty in appraising the degree of magnification. A simple standard of measurement present in many preparations is the diameter of the erythrocyte and by it the size of other structures can be approximated when necessary.

We have found it advisable to spend the first few weeks in the laboratory in a first-hand study of the varieties of the different tissue groups. The student is then given sections of organs and taught to recognize the different tissues represented in them. Then the tissue composition of organs is formally taken up. In a short time, the student is quite independent of assistance from the instructor, and this is as it should be.

Each student should have for constant use during the semester a well-selected set of slides loaned to him at the beginning of the term and returnable at the end of the course. There should be available a number of reference books and a complete set of lantern slides of drawings and photomicrographs of preparations for use during the laboratory sessions. Projection of microscopic preparations is a useful device for recitation purposes. Drawings are suggested, not as artistic representations but as pictorial descriptions of what the student actually sees. Unknown preparations are used frequently to test the student's knowledge of tissues and organs and his ability to apply it. At every step, the student is confronted with new problems requiring observation and the exercise of judgment in making decisions in identifying tissues or organs. Such mental exercise is valuable training.

Part of the time the student will spend in learning to make preparations of the various organs studied, employing the more general methods found by experience to be most satisfactory. Mastery of technique is a worthwhile educational objective. There should be available for constant reference one or more of the textbooks listed under the chapter dealing with technique, so that the student may extend his knowledge of methods as time permits.

The authors hope to have the privilege of being made aware of the criticisms and suggestions which will undoubtedly arise in the minds of those who use the book.

New York City.

G. G. S.
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CONTENTS.

CHAPTER I.

INTRODUCTION.

CHAPTER II.

THE EPITHELIAL TISSUES.

CHAPTER III.

The Connective Tissues.

CHAPTER IV.

The Blood.

CHAPTER V.

THE MUSCLE TISSUES.

CHAPTER VI.

THE NERVE TISSUE.

CHAPTER VII.

THE VASCULAR SYSTEM.

CHAPTER VIII.

THE LYMPHATIC SYSTEM.

CHAPTER IX.

The Integument.

CHAPTER X.

The Respiratory System.

CHAPTER XI.

The Digestive System.

CHAPTER XII.

The Excretory System.

CHAPTER XIII.

The Female Reproductive System.

CHAPTER XIV.

The Male Reproductive System.

CHAPTER XV.

The Endocrine Glands.

CHAPTER XVI.

Technique.

APPENDIX.

MICROSCOPIC ANATOMY OF VERTEBRATES.

CHAPTER I.

INTRODUCTION.

In courses of animal biology, or zoölogy, students usually begin by studying the Protozoa where a single cell constitutes an individual possessing a complete organization and carrying on all functions essential to life. This conception of cellular independence and self-sufficiency is modified upon consideration of simple multicellular animals, such as are found among the Cœlenterates. In such animals a large number of cells are interdependent, their functions finding expression in the activity of the individual they constitute. Cellular specialization becomes evident; certain cells become particularly active in secretion or in absorption; others serve for protection; still others function in contraction or conduction of stimuli. Among the higher groups of animals cellular differentiation and interdependence becomes more marked, so that within the individual animal body there are organizations of cells having the same structural and functional features. Such organizations of cells, structurally similar, and their products, if any, are called tissues. Five different types of tissues are recognized on the basis of the structural and functional characteristics of the cells and cellular products composing them. These are epithelium, connective tissue, muscle, nerve and blood.

Histology concerns itself with the study of the structural characteristics of tissues and their interrelationships with one another. The five types of tissue do not exist independently, but are associated in the formation and function of various organs. A better appreciation of how organs function is possible after a study of their tissue composition or microscopic anatomy. Histology and microscopic anatomy are but continuations of anatomy which concerns itself with the internal and external structures of the animal body as determined by dissection. The close association of structure and function obliges a student of anatomy to consider various

aspects of function with relation to the structural features, although this study is primarily the problem of physiology.

The study of histology and microscopic anatomy rests upon the structural and functional differentiations of the cells. Although the knowledge founded on studies of the protoplasmic structure and function of cells belongs to the science of cytology, it is essential that the student of histology should know some of the general structural features of the cell in order to understand the conditions met with in the tissue and organ structures with which his preparations deal. It is also important to remember that complex multicellular animals usually originate from the fusion of a single egg and a sperm. The progressive steps by which such a fertilized egg develops into a mature individual is the concern of embryology. We will later follow the proliferation of cells, at first similar in structure, through their early embryonic development up to the time when cellular differentiation becomes more apparent and the three cellular layers, the ectoderm, endoderm and mesoderm, are formed. From these three layers all the later tissues and organs are derived.

The study of the living cell is possible, but it is still far from being sufficiently practical to be used as a method for teaching. We must rely, therefore, almost entirely upon preparations of dead tissues and organs stained to accentuate their characteristic features. It is true that the methods used in such preparations subject the cells to a number of chemical and physical changes, but the features shown by such methods have been repeatedly checked so that in knowing the reaction of cells to these procedures we have relatively constant factors with which to deal. In the living cell many of the structures easily seen in prepared material are visible only with great difficulty. Although the cellular structures and intercellular material appearing in our preparations may be called artifacts to some extent, they will give reliable information regarding characteristic structures and reactions of tissues, providing we are acquainted beforehand with the methods of preparation used.

THE CELL.

Although there is great variation in the size, shape, and particular functions of different cells, there are certain general structural features that can usually be demonstrated in all. An animal cell may be defined as a small mass of protoplasm externally limited by

a cell membrane and containing a spherical body, the nucleus, enclosed in its own membrane. The body of the cell, or cytosome, includes all the protoplasm outside the nucleus, both nucleus and cytosome being composed of protoplasm whose composition differs in each case.

Protoplasm.— Protoplasm is chemically a complex mixture of proteins and their derivatives, carbohydrates, lipoids, and inorganic salts associated with a large amount of water. Physically, protoplasm has the properties of a complex colloidal system, capable of those changes in viscosity which are due to reversible gels occurring in the living cell. Living cells are visible as pale, colorless, homogeneous masses in which, with proper illumination, various refractive structures may be observed. The cell membrane and the nuclear membrane may appear brighter because of the higher refractivity of their components. Likewise the chromosomes of the nucleus, together with some of the formed granular or fibrillar elements of the cytosome, may stand out. For the most part, our knowledge must depend upon examination of fixed and stained material in which the various elements of the protoplasm are precipitated as insoluble substances which are then stained with various dyes. The precipitation process also preserves the membranes; and, depending upon the reagents used, other structures of the cytosome, such as the mitochondria, or chondriosomes, Golgi apparatus, and centrioles, may be precipitated for staining and study. (Fig. 1.)

Cytosome.—The appearance of the cytoplasm in fixed materials varies with the method used in preparation, and also with the type and physiological state of the cell. It was this variability with fixatives which caused much of the early disagreement and debate among scientists as to the nature of protoplasm, since each based his conception on his prepared material. Accordingly, some investigators held that protoplasm was essentially granular; others believed it to be alveolar and to resemble an emulsion; still others held that it had a reticulum of slender fibrils associated with fine granules. Actually all these conditions may be observed in the same type of cell, if it be treated with various techniques. However, certain definite structures or organelles are differentiated in the cytosome.

Chondriosomes, or mitochondria, are elements demonstrated within the cytoplasm after certain techniques. They are quite universal in occurrence, though variable in shape. Usually they are granular in young or embryonic cells but filamentous or rod-

shaped in the older tissue cells.   They are composed of phospho-
lipids and protein in varying proportions, and are soluble in fat
solvents, but can be preserved by formalin and potassium dichro-
mate.   Janus green reacts with them in the living cell, and their
activities may be readily followed after staining with this dye.
Various observers have associated them with the formation of fat,
of fibrils, and of secretion granules.   They move about in the
cytoplasm, grow and divide, and transform from the granular to
the rod or filamentous forms (or *vice versa*) in the living cell.
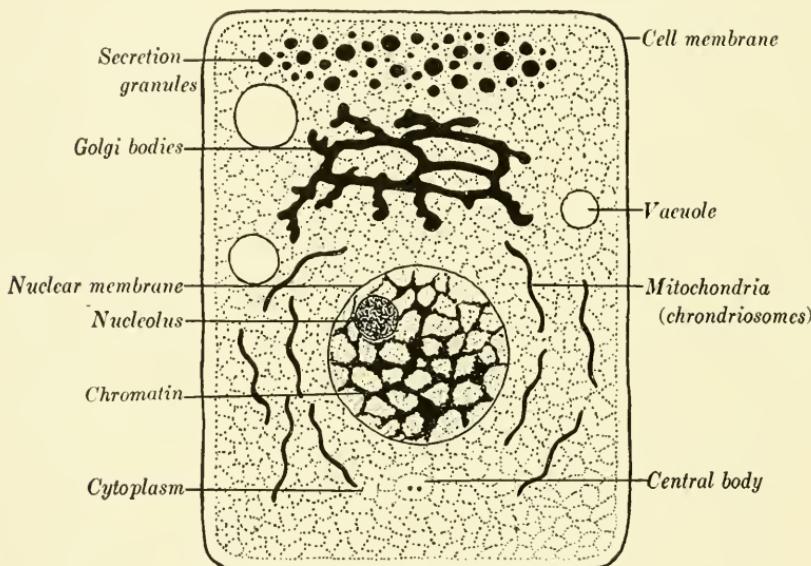
Fig. 1.—Diagram of a typical cell.

The apparatus of Golgi, a reticular structure often close to the
nucleus, was first demonstrated in certain nerve cells.   These bodies
are formed of lipoids, which are fixed with osmium tetroxide, or
they may be dissolved with fat solvents, leaving empty canaliculi
in their place.   Their function is unknown, but their occurrence
is so general that they are considered normal intracellular structures,
concerned in some unknown manner with cell function.   They
apparently vary in composition with different states of the cell.

Close to the nucleus there is to be found another structure of
almost universal occurrence in animal cells, the central apparatus,
or centrosome.   In the resting cell it is inconspicuous, but becomes
prominent during mitotic (indirect) division of the cell.   During

this process, a single small granular body, the centriole, is surrounded by a clear area of cytoplasm, the centrosphere. After treatment with protein-precipitating agents, numerous fine protoplasmic rays may be made out, extending in all directions into the cytoplasm from the centrosphere. The so-called astrosphere thus formed is not commonly visible in living material nor in material otherwise fixed. The function of this apparatus is not clearly understood, but appears to be intimately connected with mitosis.

In addition to the bodies already mentioned, there are other less common particles distributed in the cytoplasm. In nerve cells and striated muscle fibers, abundant and distinct fibrils may occupy much of the cytosome. The cells in the early divisions of the frog embryo contain yolk granules, and glycogen can be demonstrated as a stored substance in the cytoplasm of liver cells. Under favorable conditions, droplets of fat form in certain connective-tissue cells. Granules of pre-enzymatic nature are recognizable in gland cells, and pigment granules occur in the cytoplasm of certain epithelial and connective-tissue cells.

A limiting membrane is not clearly evident in all cells. Some investigators believe it to be merely a condensation of the peripheral cytoplasm which probably contains lipins. By using microdissection apparatus it has been possible to indicate that this membrane possesses some degree of elasticity. It has been demonstrated to be semipermeable and appears to regulate the passage of oxygen and nutritive materials into the cell cytoplasm as well as the passage of carbon dioxide and nitrogenous wastes out of the cell. The exposed surface of some cells may have a thick, tough external cuticle or other structural modifications. Certain groupings of cells are not completely separated by individual cell membranes and form a syncytium as in the case of cardiac and skeletal muscle.

Nucleus. — In general, the nucleus of the cell is a globular or ovoid body and is separated from the cytoplasm by a definite membrane. Within the membrane, the nucleus is composed of a homogeneous watery fluid, containing special proteins, the nucleoproteins or chromatin in colloidal solution. Fixation and use of basic stains bring out a granular network of chromophilic substance that increases in density as organization of the nucleus for division advances, until the chromosomes, which are mainly solidly staining bodies composed of nucleic acid, stand out clearly. Cytogenetics has demonstrated that it is the chromatin material of the nucleus which carries the hereditary factors. Nucleus and cytoplasm differ

2

in their reactions to stains. Cytoplasm takes acid stains, therefore staining red with eosin. The chromatin of the nucleus reacts mainly to basic stains so that when hematoxylin is employed the chromatin has a color varying from blue to purplish-black. In the resting nucleus the chromosomes are not apparent. Their disappearance is presumably due to the incorporation of basic proteins in the nucleic acid molecule, which then goes into solution. Two types of chromatin are thus indicated in preparations of cells in the resting state; oxychromatin, which has an affinity for acid dyes; and basic chromatin with an affinity for basic dyes.

Within the nucleus of the resting cell there is usually present at least one small spherical body, the nucleolus, that in living cells is highly refractive. This body has an affinity for acid dyes, but may also stain with basic dyes and resemble chromatin in appearance. Its function is not definitely known, but there are some indications that it may be associated with the storage of nuclear wastes and of chromatin. It usually disappears during the mitotic process.

The nucleus is regarded as being the regulator of cellular activities, and changes in its appearance and reactions are associated with the changing states of the cell as a whole. One of the major functions in which the nucleus is intimately involved is cellular division, or reproduction.

Cell Reproduction.—The formation of new cells is most active in the embryo, yet the process takes place in many tissues of the adult vertebrate body notwithstanding the fact that there are surprisingly few cells in the active process of division to be seen in the usual microscopic preparations. Probably most of the cells of which adult tissues are composed have a longer life than one would at first suppose. We should think of a cell as a complicated biochemico-physical system whose organization is maintained for a long time, even though the materials of the system may change.

Two types of cell division have been recognized, mitosis and amitosis. Mitosis, or indirect cell division, is the normal method of cell division, and a brief presentation of this process may serve to recall the major features.

Mitosis.—The mitotic process is usually arbitrarily divided into four stages: namely, prophase, metaphase, anaphase, and telophase. These imperceptibly grade into one another, since they are but easily distinguishable steps in a continuous sequence of phenomena.

Prophase.— This phase of nuclear activity is preceded and accompanied by activity of the two centrioles in the center of the cytocentrum or attraction sphere. When activity begins, the centrioles move apart until they occupy a position opposite each other 90 degrees from their resting position, with the nucleus between them. Astral rays appear in the cytoplasm, radiating out from each centriole. In preparations, these rays often resemble fibers. Some of them form a fibrillar apparatus between the two centrioles, which is known as the achromatic spindle, or, together with the centrioles, the amphiaster. Simultaneously the nuclear membrane and nucleolus disappear, and nucleoplasm mingles with cytoplasm. The distributed chromatin granules of the resting nucleus are apparently organized into a long, coiled, tangled thread, which shortens through condensation of its constituents and finally forms a specific number of pieces, or chromosomes. The number of chromosomes forming at this stage is usually constant for all somatic cells of each species. The chromosomes move so that they lie on the spindle in an imaginary plane midway between the two centrioles at right angles to the axis. Longitudinal splits in the chromosomes now become apparent as grooves, though they may actually have split much earlier in the prophase stage, and some of the astral rays (fibers) appear to attach to chromosomes.

Metaphase.— During this second phase longitudinal splitting is completed and each chromosome is found to be equally divided into two longitudinal halves. The two halves of each chromosome separate as they move toward their respective centrioles, a feature marking the beginning of the anaphase.

Anaphase.— In this third stage, the chromosomes continue toward the centrioles. In late anaphase a groove appears in the cell membrane corresponding with the location of the equatorial plane, which is marked by a granular line. This groove deepens as the chromosomes pass to opposite poles. The chromosomes assemble at opposite poles and initiate the telophase stage.

Telophase.— In the final phase the equatorial constriction, or groove, continues so that eventually 2 daughter cells are formed, each with its own cell membrane. Two daughter nuclei are organized in the same way as was the original resting nucleus, though they are smaller in size. In this process, a nuclear membrane forms about each daughter nucleus, and the chromatin becomes distributed in it as it was in the resting nucleus of the parent cell. A nucleolus

usually appears in each nucleus at the end of the telophase. Also, by this time, the centriole has divided into a diplosome, or 2 centrioles, near each daughter nucleus. If the daughter cells chance to be part of a very young embryo, the next cell division will occur very quickly. But if the cells are part of an adult organism, some time may elapse before they divide.

AMITOSIS.—Now and then cells appear to divide directly by constriction without the elaborate preparations occurring in mitosis. This process has been named amitosis. In such cases the nucleus first separates into two parts by constriction, after which the cytoplasm likewise divides. It has been observed in pathological tissues and in the case of some old cells in tissue cultures. In most of the so-called cases of amitosis, the technique may have been at fault or the cells only appear to divide amitotically, due to some abnormal condition interfering with the mitotic process.

Cell Metabolism.—The living cell is a dynamic system in which chemical and physical changes are constantly taking place. All such transformations of matter and energy occurring in living protoplasm are designated by the term metabolism. In this term are included the anabolic processes involved in building tissues up as well as the katabolic or breakdown processes. Nutritive substances taken into the cell are subjected to the action of enzymes which facilitate the breaking down of the complex compounds into their simpler units. These units are then utilized in building up the elements of protoplasm which serve for repair and growth. The energy liberated by oxidation reactions is utilized for the chemical and physical changes involved in movement, in secretion and excretion, and for the continuation of those metabolic changes constantly involved in the maintenance of life.

All cells regardless of the degree of their functional specialization must still carry on a basic general metabolism necessary to maintain their own life. Thus a muscle cell, in addition to the basic metabolism maintaining its life, has special metabolic processes endowing it with the property of contraction.

Cytomorphosis designates the structural changes undergone by a cell in its passage from the embryonic state, through its differentiated phase to senescence and death. Death or necrosis of cells is followed by cellular disintegration, at which time the enzymes active in the living cell in the synthesis of proteins from amino-acids begin breaking down the proteins into amino-acids which are then diffused from the cell. During this process of autolysis the chromatin of the nucleus contracts into a dense irregular chromatic mass.

Tissue Culture. One of the achievements of modern research has been the development of methods whereby small fragments of tissues composed of various cells can be isolated and grown apart from the organism in especially prepared media. If proper conditions of warmth, aëration, and asepsis are maintained in the culture media, the cells of such tissue cultures may be kept alive, grow, and divide mitotically over a considerable period and a number of generations. It has been possible by these methods to isolate certain cell types, observe their growth, and determine whether they maintain certain characteristics or transform to other types. It also opens a method of attack upon the physical and chemical properties of the various cells maintained under culture conditions. One of the outstanding contributions has been the study of growth and regeneration of nerve cells. Still other observers have studied the behavior of other types of cells under different physical and chemical experimental conditions.

Another method of attacking the study of living cells utilizes a micromanipulating apparatus in conjunction with the microscope. By means of this apparatus those proficient in the technique have been able to dissect cells or inject minute quantities of chemicals into them. As a result of these microdissections and microchemical studies much has been learned about the physical and chemical nature of living cells.

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EMBRYOLOGY.

A vertebrate normally begins life as a single cell, the zygote, which is formed by fusion of a sperm cell and an ovum in the process of fertilization. Almost immediately the zygote begins dividing by mitosis to form an increasing number of cells of decreasing size. Each cell so formed in these early divisions is called a blastomere, and the process itself is termed cleavage. The amount of yolk originally stored in the ovum varies in different classes of vertebrates, and since the presence of yolk modifies cleavage and later developmental stages by hindering mitotic activity where it is abundant, the morphology of later stages varies. Nevertheless, homologous stages in development can be recognized.

Amphioxus.—The relatively simple development of Amphioxus is usually studied as exemplifying clearly the early stages, since there
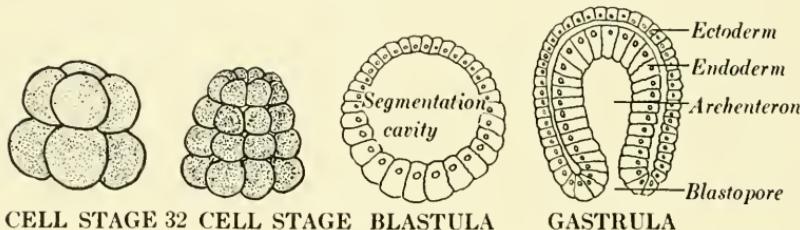
8 CELL STAGE   32 CELL STAGE   BLASTULA   GASTRULA

FIG. 2.—Diagram of early stages in development of Amphioxus. (Modified from Arey's Developmental Anatomy, W. B. Saunders Company.)

is only a very small amount of yolk stored in the ovum. The yolk present is localized toward one end of the cell, known as the vegetal pole, and at the opposite end, the animal pole, protoplasm is more concentrated. However, the yolk is so small in amount that mitotic activity is little affected and nearly equal blastomeres are formed by cleavage. As mitosis continues, the cells become more numerous and smaller, those toward the vegetal pole becoming slightly larger than those at the animal pole. As the cells continue to divide, they maintain a peripheral position and enclose a central cavity. (Fig. 2.) Thus a hollow sphere, the *blastula*, is formed, with its wall composed of a single layer of very small cells enclosing a central segmentation cavity filled with liquid. Mitotic activity continues following blastula formation, and the slightly larger vegetal cells are folded into the segmentation cavity. Invagination of these cells continues to form an inner layer adjoining the outer cell layer and practically obliterating the segmentation cavity, thus

forming a gastrula. The opening at the point where the invagination of vegetal cells occurred is called the *blastopore*. The outer layer of cells composes the ectoderm; the inner layer is the endoderm; and the cavity within the endoderm layer of cells is the *archenteron*, or primitive gut, which opens to the exterior by the blastopore.

A third germ layer, the mesoderm, makes its initial appearance from a series of paired pouch-like evaginations along each side of the dorso-lateral wall of the endoderm of the primitive gut. These evaginations, the cœlomic pouches, later separate from the endoderm. The cœlomic pouches on either side enlarge and grow ventrally around the gut, the transverse walls of adjacent pouches fuse and are obliterated so that a common cavity, the cœlome, is formed. The outer wall of the mesoderm adjacent to ectoderm is known as somatic mesoderm and together with the ectoderm is called the somatopleure, which gives rise to the body wall. The inner layer

8 CELL STAGE   BLASTULA   GASTRULA
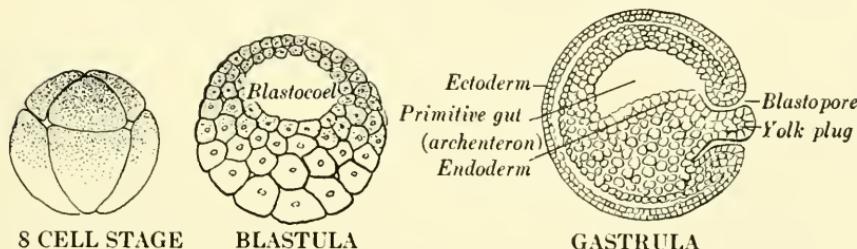
Fig. 3.—Diagram of early stages in development of the frog.

of mesoderm adjacent to the endoderm of the gut wall is known as splanchnic mesoderm and together with the endoderm composes splanchnopleure, which gives rise to the gut wall.

Amphibia.—In this group, as exemplified by the frog, the eggs contain considerable yolk. The nucleus and bulk of the cytoplasm are restricted to the animal pole and the abundant yolk is concentrated toward the vegetal pole. Cleavage is complete but the cells are of unequal size, those toward the vegetal pole being distinctly larger than those of the animal pole. The segmentation cavity of the blastula is reduced in size with yolk-laden cells on its floor. (Fig. 3.) Gastrulation is atypical, invagination of vegetal cells being limited, and the gastrula appears to be formed as a result of overgrowth and infolding of the more actively dividing cells of the animal pole in the region of the blastopore. The gastrula is similar to that of Amphioxus, except for the larger yolk-laden cells below the floor of the archenteron.

The dorsal wall of the archenteron consists of relatively few layers of small endoderm cells, while the floor consists of many layers of large yolk-laden cells. Certain cells along the dorsal mid-line differentiate into a rod-like structure, the notochord, and other cells proliferate off on either side of the notochordal region to form mesoderm masses separated from ectoderm and endoderm. Eventually the right and left sheets meet along the mid-ventral line and fuse.

Before this fusion occurs, the mesoderm mass begins to split into two sheets on each side a short distance away from the notochord. This continues so that eventually, as in Amphioxus, there will be an outer somatic sheet associated with ectoderm and an inner splanchnic sheet associated with endoderm. The space between them is the cœlome, or body cavity.
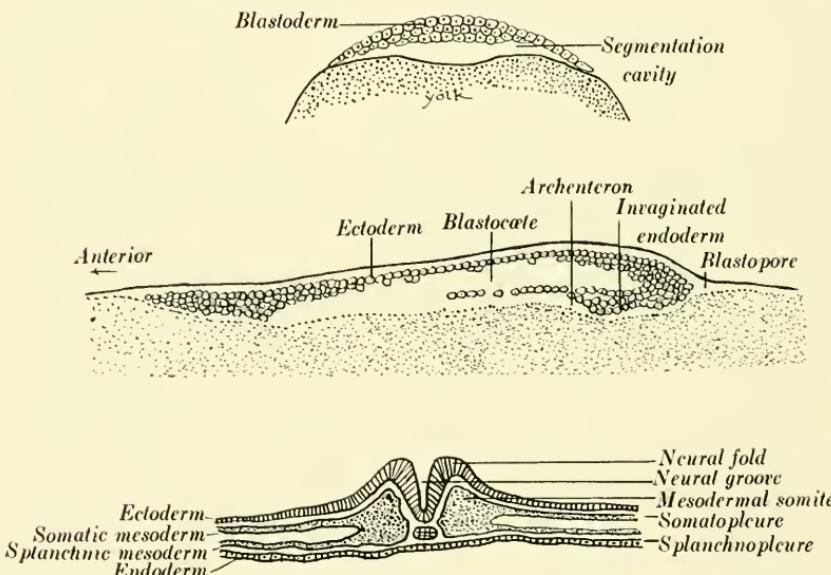
Fig. 4.—Diagram of early stages in development of reptiles and birds. (Modified from Arey's Developmental Anatomy, W. B. Saunders Company.)

Sauropsida.— In reptiles and birds, where the egg contains a large amount of yolk, the nucleus with a small amount of cytoplasm is located at the animal pole. This restricted protoplasmic region divides into cells, but the larger yolk portion takes no part in cleavage. Cleavage is therefore partial and results in a small disc of cells on top of the spherical yolk mass. This disc of cells, called a blastoderm, continues to divide by a series of vertical and horizontal planes, so that in the late cleavage stage there are a few layers in the central part of the disc, but only a single layer of cells

at the margin. A small space between the blastoderm and the yolk represents the segmentation cavity. Additional marginal cells continue to form in such a manner that they eventually surround the yolk, but never take part in the formation of the embryo itself. Gastrulation is accomplished by an ingrowth of cells along the posterior margin of the blastoderm. (Fig. 4.) Following endoderm formation, a primitive streak appears on the blastoderm as a linear thickening in which a shallow depression, the primitive groove, appears. This groove is believed to be homologous with the blasto-pore of Amphioxus. Mesoderm arises from lateral proliferations of cells of the primitive streak which form plates that grow laterally between the ectoderm and endoderm on either side of the mid-line. The plates split distally, thus forming the somatopleure and the splanchopleure with ectoderm and endoderm, respectively. The cœlome is the space between somatic and splanchnic mesoderm.

Mammalia.—Although the eggs of mammals possess a minimum of yolk, the development of the embryo does not follow the simple plan outlined for the egg of Amphioxus. Development of the mammal is complex and possibly modified because of evolution from an ancestral condition in which yolk was abundant in the egg, a condition still present in Monotremes. Yolk has practically dis-appeared from most mammalian eggs and the zygote developing within the parental body secures nutritional supplies from maternal tissues. Such modifications change the developmental processes by which the embryo becomes established.

Cleavage is complete and the early stages superficially resemble those of Amphioxus. Later cleavage results in the formation of an inner cell mass surrounded by a single layer of cells, called the trophectoderm, which is in contact with the wall of the uterus of the mother.

The trophectoderm grows more rapidly than the inner cell mass which is left attached only at one point on the trophectoderm wall. The cavity between the two regions is filled with fluid and is homolo-gous with the segmentation cavity of lower forms. This stage is recognized as a specialized blastula and is called a *blastocyst*. The embryo proper develops from the inner cell mass which is homolo-gous with the disc-like blastoderm of the reptiles and birds. Cells derived from the lower surface of the inner cell mass form endoderm and the remainder of the mass becomes ectoderm.

Eventually a primitive streak appears on the blastocyst and is morphologically similar to that in Sauropsida. A primitive groove also appears which is homologous with the blastopore of lower

forms. Mesoderm forms on either side of the primitive streak and grows into the space between the ectoderm and the endoderm, as in preceding groups. The mesoderm mass splits into two sheets, a somatic layer associated with ectoderm to form somatopleure and a splanchnic layer associated with endoderm to form splanchnopleure.

Organogeny.—The development of similar organs differs in details in different classes of vertebrates and a complete account belongs to the field of comparative embryology. Later, as each tissue and system of organs is considered, reference to early embryology will be made wherever it is considered essential to an understanding of the microanatomy of the organ in question. The following table summarizes the structures usually considered as developing from ectoderm, mesoderm and endoderm:

From Ectoderm.	From Mesoderm.	From Endoderm.
Epidermis of skin, hairs, nails, sweat and sebaceous glands, claws, superficial portion of feathers, arrector pili muscles in skin; lens of eye; part of cornea; retina; conjunctiva; lacrimal gland; most of pituitary body; epithelium lining mouth and nasal cavities; sensory cells of many sense organs; pineal gland; medulla of adrenal gland; enamel of teeth; all nerve cells and most neuroglia cells.	Cartilage, bone, perichondrium, periosteum, tendons, ligaments; other connective tissues; microglia cells; almost all muscle tissue; blood cells; marrow; blood vessels and lymph vessels; gonads and kidneys; gonaducts and urinary ducts; cortex of adrenals; pleurae; pericardium; peritoneum; lymph organs; scales in fishes and bony plates in reptiles; major part of feathers.	Epithelium of digestive tract from pharynx to rectum; glands of this tract, including pancreas and liver; epithelium of larynx, trachea, and lungs; glandular epithelium of thyroid and parathyroid glands; epithelium lining the Eustachian tube and middle ear.

In the past it has been a common practice to emphasize the origin of the tissues and organs with regard to the germ layers, but embryological studies show considerable variations with regard to such origins. Until the embryologists have settled the debatable points we are not justified in placing much emphasis on origins.

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THE EPITHELIAL TISSUES.

THE various types of epithelial tissues are composed of one or more layers of cells lying so close to one another that there is practically no intercellular material. Membranes of this tissue cover the free surfaces of the body and line not only the ducts connecting with the surface, but also cavities within the body not connected with the outer surface. One of the chief general functions of these tissues is protection, such as preventing the loss of body fluids or the invasion of foreign material. Most types of epithelia have some secretory activity, but those lining numerous glands are primarily secretory. Epithelia are intimately concerned in processes involved in respiration, in the assimilation of nutritive materials, and in the elimination of wastes.

Classification.—It must be admitted that any classification of the various types of epithelia will be artificial, and the justification of any scheme will lie in the ease and completeness it offers for dealing with the types in question. A classification of epithelia is not possible on the basis of function alone, for the exact function of many types is not yet clear, and others may have more than a single function. Embryological origin likewise fails, since often the same type is derived from ectoderm, endoderm, and mesoderm. The most satisfactory scheme uses the form and arrangement of the cells. Even on this basis, allowances must be made for intergrading forms. Two large groups are separated depending upon the presence of one or more than one layer of cells in the tissue. Tissues belonging to the first group have a single layer of cells and are called simple epithelia. In the second group are the stratified epithelia, where more than a single layer of cells compose the tissue. Various types within these two groups are then separated with reference to the form and arrangement of the cells composing each.

SIMPLE EPITHELIA.

This group is usually subdivided into three types, namely, squamous, cuboidal, and columnar. (Fig. 5.) These terms are convenient for indicating types commonly appearing in histological

preparations, but between them there exist numerous intermediate forms. Those cells having the form resembling scales or irregular discs are called squamous. In other cells the three dimensions are approximately equal and these are called cuboidal epithelial cells, although they are rarely perfect cubes. The columnar type has elongate prismatic or roughly hexagonal cells that resemble irregular columns.

It is unfortunate that in the study of histological preparations the field presented by the microscope is primarily two-dimensional. The third dimensional aspect of the objects under study is dependent upon the focus, and in the case of very thin sections it is necessary to study a series of consecutive sections and develop the ability to form a composite picture incorporating this aspect. As an example of limitation imposed by studying the two dimensions presented by histological preparations under the microscope, consider the possible sections through a cell having the form of a

SQUAMOUS   CUBOIDAL   COLUMNAR
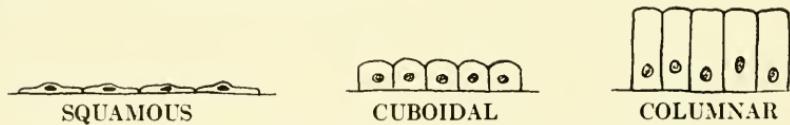
Fig. 5.—Diagram of types of simple epithelial cells.

hexagonal column with a central bean-shaped nucleus. (Fig. 6.) If a series of cuts pass through such a cell parallel with the long axis, thin sections will be obtained which appear as rectangles, and not all sections will have the nucleus represented. Furthermore, if the first cut through the region of the nucleus takes only a small portion of its convex surface, such a section will appear to have a spherical nucleus, while sections through the body of the nucleus will have an ovoid form. No one section so obtained gives any clear evidence of the third dimensional aspect or the picture of the cell as a whole. If another series of thin sections is obtained using cuts at an angle of 45 degrees with the long axis, then there will be a variation from trapezoids without nuclear portions to rectangles containing roughly spherical nuclear portions. As the angle of cutting approaches a right angle with the long axis, the hexagonal structure of the cell appears in the sections, although distorted. Sections from cuts made at right angles to the longitidinal axis will show hexagons, some of which will have no nuclear portion, while others will have a spherical nucleus centrally situated. Therefore, in order to visualize the complete structure of such a

cell it would be necessary to have at least several sections at right angles to and parallel with the long axis. In studying tissues and organs, an attempt should be made to visualize the third dimensional aspect and consider the relationship of the cells with each other. In the example just considered the cell was a regular, independent unit, but as a part of tissues all cells are modified by pressures of adjoining cells and internal pressures, so that the common form is an irregular prism which in sections presents polygonal figures. In
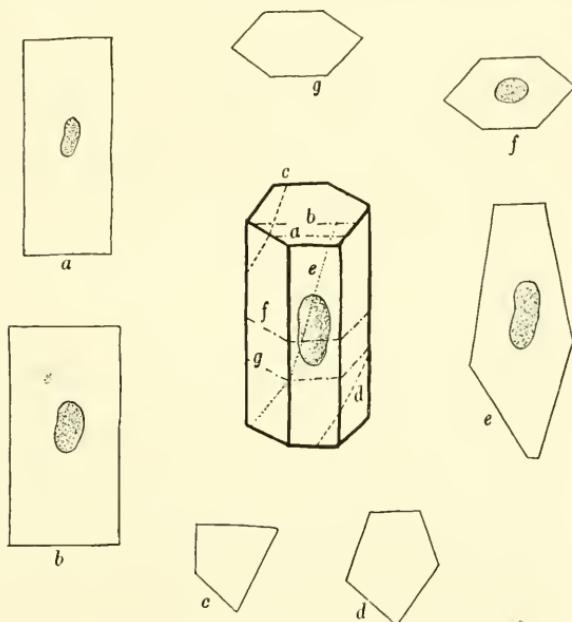
Fig. 6.—Diagrams of the appearance of a variety of sections of a typical columnar epithelial cell.

general, those cells which appear as squares in sections passing through their central region at right angles to their base are spoken of as cuboidal, but usually they are iso-prismatic in form. Likewise, cells appearing as rectangles in sections passing at right angles to their base are spoken of as columnar cells, but are long prismatic structures when the third dimensional aspect is considered. The squamous cells appear as fusiform structures in sections resulting from cutting at right angles to their basal or attached surface. The three types indicated show great variation as a result of irregularities imposed by adjacent cells and the location of the tissue so that inter-grading types are common.

Simple Squamous Epithelium.—The term squamous literally means scaly, and epithelia of this type are composed of flattened, plate-like cells whose cytoplasm is so scanty that the nucleus, which is
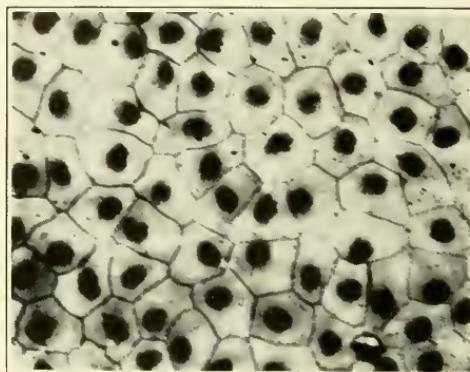
FIG. 7.—A photograph of a surface view of simple squamous cells forming the top-most layer of a frog's epidermis.
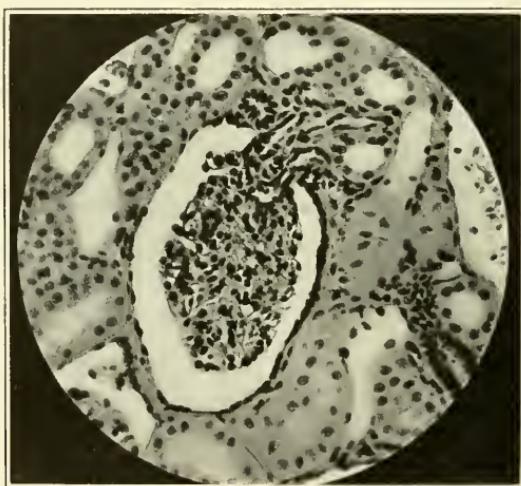
FIG. 8.—Photograph of a section through the kidney of a frog. Simple squamous epithelial cells are shown lining the space surrounding the glomerulus. Cuboidal cells form the walls of the surrounding sections of uriniferous tubules. The arterial vessels are shown entering the glomerulus at the top.

centrally placed, causes a slight bulge. (Fig. 7.) In some cases the lateral faces or edges adjoining other cells are regular, but more often the boundaries are irregular and interlock with irregularities in adjacent cells. In sections, which may pass at right or even

oblique angles to their bases, such cells appear as fusiform when the central nuclear region is represented, or they appear as a very narrow band with indistinct cellular outlines when sections miss the nuclear region. A simple squamous epithelium is found (Fig. 8), for example, forming Bowman's capsule of the uriniferous tubules of the kidney.
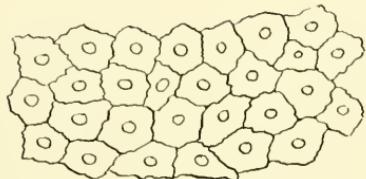
FIG. 9.—Diagram of mesothelium.

FIG. 10.—Diagram of endothelium.

A membrane of squamous cells with wavy, irregular boundaries forms the surface layer of the mesentery and the peritoneum of mammals. (Fig. 9.) It is called mesothelium and very commonly its cells have cilia from their free border.

FIG. 11.—Photograph of simple cuboidal epithelium of a collecting tubule of the rat kidney. Surrounding it are a number of capillaries with walls composed of simple squamous cells.

The cavities of the heart and all blood and lymph vessels (Fig. 10) are lined with a layer of elongated simple squamous cells, similar to those of the mesothelium. Their long axis is usually directed along the line of flow within the vessel. This simple squamous type so located is called endothelium.

By subjecting fresh mesentery to a weak bath of silver nitrate and bright sunlight, a deposit of silver salt occurs between adjoining cells and the cell boundaries stand out clearly. In this way it is possible to show the larger mesothelial cells with wavy outlines and the narrower and longer endothelial cells forming the lining of small vessels.

Simple Cuboidal Epithelium. — In this type the epithelial membrane is composed of cells whose three dimensions are approximately equal and appear in sections as rough squares. Cuboidal epithelium is found in portions of the excretory ducts of many glands and in certain areas of the uriniferous tubules of the kidney. (Fig. 11.)
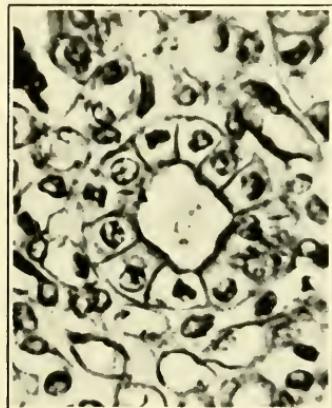
It is also found covering the mesentery and peritoneum in many
lower vertebrates in place of the simple squamous commonly found
in mammals. When arranged about small ducts the cells are usu-
ally somewhat truncated with the free end smaller than the base.
Cuboidal cells in some situations, as in parts of the kidney tubules,
are often ciliated.

A cuboidal epithelium is also found covering the ovary of
mammals, where it is spoken of as a germinal epithelium, though it
normally takes no part in germinal activity of the mature ovary.

Simple Columnar Epithelium.—The cells of this type show varia-
tions from those whose length is only slightly greater than their
other two dimensions to cells which are greatly elongated. (Fig. 12.)
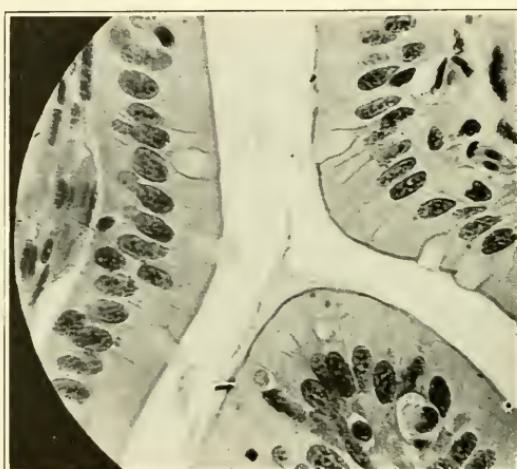
Fig. 12.—Photograph of simple columnar epithelial cells forming the lining of
the intestine of Necturus. Several goblet cells are shown with their apical portions
filled with mucous secretion.

The nuclei may occur in the central region of such cells, but more
commonly are found in the lower half. The low columnar cells
closely resemble the cuboidal and it is often difficult to decide
whether to call a given type cuboidal or low columnar unless a
number of cells can be observed. In sections where the cut has
been parallel to the long axis, columnar cells appear as rectangles,
but sections at right angles to this axis show irregularly polygonal
figures. When serving to form ducts these cells may taper from
base to apex, forming an elongated prism, or roughly pyramidal
figure in section. Epithelium of a columnar form is found

lining the digestive tract from stomach to anus in mammals, and lines the many small glands in the wall of the stomach and intestine of vertebrates generally, as well as some of the duct systems of the larger digestive glands, such as the liver, pancreas, and salivary glands.

The epithelial sheet in the alimentary tract of many lower vertebrates is composed of very long cells whose tapering basal portions extend between shorter, irregularly fusiform cells. Although all the cells rest upon the same base, there is the appearance of a number of layers present. Even in the more typical columnar epithelia there are a number of smaller polyhedral, fusiform, or spherical cells, often intercalated basally. This brings us to the term pseudostratified epithelium, which is commonly used for an epithelium falsely resembling the stratified columnar type. (Fig. 13.)

It has the appearance of several strata of cells with columnar cells forming the layer nearest the surface. It has been shown, however, that if a section passes parallel to the long axis of this epithelial membrane all the cells will be seen to rest upon a common basement membrane adjacent to the underlying connective tissue. The cells of the superficial stratum have long, taper-

Fig. 13. — Diagram of ciliated pseudostratified epithelium.

ing basal extensions, reaching down to the basement membrane, and polyhedral cells of various sizes fill in about these slender portions and also reach to the basement membrane. Most preparations do not differentiate the lateral boundaries of the cells clearly enough to show the absence of stratification, but the rows of nuclei at different levels indicate cells of different lengths. This type, with cilia present on the free borders of the columnar cells, forms the lining of the trachea and bronchi of mammals. The epithelial membranes in the case of the lining of the alimentary tract of many lower vertebrates appears to be of the same structure but the columnar cells are longer and it is more difficult to separate the tissue into truly stratified and pseudostratified epithelia.

A simple columnar epithelium is associated with secretion and single cells of the membrane accumulate the secretion product until the free end is much distended and resembles a goblet. (Fig. 12.) Such cells are called goblet cells and occur abundantly in the lining

3

of the intestines. Other columnar cells, composing the small glands associated with the stomach and intestine, appear to be uniformly distended by their secretory activity and the passage of the secretion out of the cells is gradual and continuous, not abrupt as in the case of the goblet cells.

In a number of cases cells with a sensory function are derived from embryonic ectoderm to form parts of sensory organs. Although such cells may be classified as columnar they are commonly fusiform in shape. Associations of such cells, called neuro-epithelium, occur in the taste-buds of the tongue, the rods and cones of the retina of the eye, and in special sensory bodies in the skin of fishes and amphibians.

STRATIFIED EPITHELIA.

When considering stratified types several subdivisions are possible on the basis of the shape and arrangement of the cells, with the emphasis on the type of cell forming the superficial layer. The basal layer of cells is generally prismatic and appears as cuboidal or low columnar in the sections. The cells between the base and the surface appear as irregular polygonal figures, showing considerable variation in size, regularity, and number of layers. In some cases the cells flatten out as they near the surface, until those of the superficial layer are squamous in form; in other instances the cells retain a prismatic structure even at the surface, save that their free boundaries are usually convex; but in other cases the surface cells are elongated prisms appearing as columnar cells in sections, with tapering bases passing among the underlying polygonal cells. Four subdivisions of stratified epithelia are discussed in the following paragraphs.

Stratified Squamous Epithelium.—This is a common stratified type and the number of cell layers composing the membrane varies in different parts of the same animal. In sections, the basal layer of cells appears cuboidal or low columnar. Progressing upward there are several layers of polyhedral cells showing a gradual change toward the surface where flattened squamous cells occur. (Fig. 14.) The superficial squamous cells are constantly being worn away and replaced by underlying cells. The cells of the basal layers divide frequently and the plane of division parallels the base, so that after each division one daughter cell remains in the same position as the mother cell while the other is pushed upward into the next layer. The newly formed cells are constantly undergoing chemical

alterations as they move closer to the surface of the membrane, until finally they die and become part of the surface layers which continually wear away. Microscopic examination of saliva reveals a number of such desquamated cells from the lining of the mouth. In mammals this type is found in such structures as the lips, oral cavity, esophagus, and epidermis of the skin; there being considerable variation in the thickness of the membrane and its composition in these different regions. Although the best examples occur in mammals, stratified squamous epithelium forms the epidermis of many lower forms. In fishes and amphibians some of the cells in
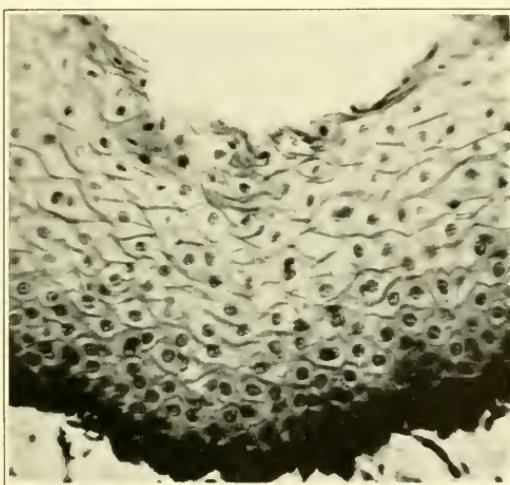
Fig. 14.—Photograph of stratified squamous epithelium from esophagus of monkey.

the epidermis become spherically distended by accumulation of secretions which are later liberated on the skin surface.

Stratified Cuboidal Epithelium.—The basal cells are cuboidal or low columnar, the intermediate region of several cell layers has irregularly polygonal cells, and the surface cells are roughly cuboidal with a rounded outer margin. This type is commonly found forming the epidermis of urodeles, where scattered cells of the intermediate layers are secretory and become distended with their secretion products until they appear almost spherical. (Fig. 90.)

Stratified Columnar Epithelium.—The basal layers and intermediate cell layers are similar to those of the preceding stratified types, but the superficial cells are elongated prisms that appear columnar in sections. It is found in mammals lining, the vas deferens and some of the larger excretory ducts of certain glands.

In some cases, as in the vas deferens, the columnar cells may be ciliated. Among the lower vertebrates this type seems to be more commonly distributed, although many cases so distinguished may prove to be a pseudostratified arrangement such as we have already mentioned. It appears in the alimentary tract of many lower forms and in the excretory ducts. In some regions, as in the esophagus of many amphibians (Fig. 15), some of the columnar superficial cells are ciliated, and others are transformed into goblet cells filled with mucin.
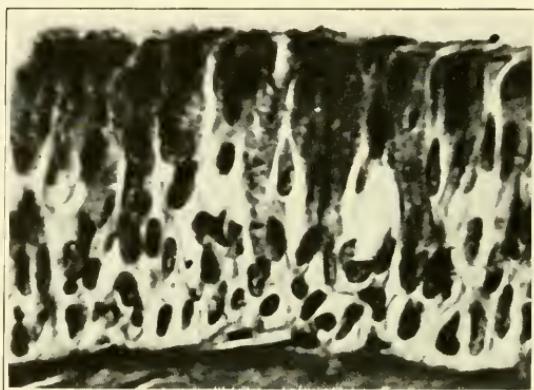
FIG. 15.—Stratified ciliated columnar epithelium from the frog's esophagus. Note the long superficial cells and several layers of small basal cells.

Transitional Epithelium.— In this type the basal layer of cells is cuboidal or low columnar in appearance in sections, while at the surface of the membrane are very large cells convex on their free boundary and with concavities on their lower surface into which underlying polyhedral cells fit. Binucleated cells commonly occur in this tissue. Transitional epithelium lines the pelvis of the kidney, the ureters, the bladder and part of the urethra of mammals, in which group it appears to be well developed. Its cells seem to be capable of considerable displacement; they slide by one another under tension so that in a distended membrane of this type there appear to be only two or three layers of cells. The surface cells stretch most and the underlying cells are drawn out into a single or double layer beneath them; as the tension on the membrane is released the cells slide back, until the stratified appearance of a number of layers is resumed. Membranes of this type show gradations to those resembling a stratified cuboidal or stratified columnar, conditions commonly occurring in the urethra. (Fig. 130.)

PIGMENTATION IN EPITHELIAL CELLS.

In various locations epithelial cells normally contain pigment granules, and this constant characteristic has lead to speaking of such epithelia as being of the pigmented type. However, this does not indicate the morphology of the cells, which may be rightly classified as cuboidal or other types. To follow the scheme of morphology in classification, such cells should be classified on the basis of their form and arrangement and then the presence of the pigment should be noted for the given type in certain locations. An example of this tissue occurs in the outer layer of the retina, where hexagonal cells adjacent to the inner surface of the choroid coat are heavily pigmented. Pigmentation granules also occur in the lower layers of cells in stratified squamous epithelium forming the epidermis of various mammals and in the liver cells of many lower forms.

SURFACE MODIFICATIONS OF EPITHELIAL CELLS.

The free boundaries of epithelial cells may be modified in various ways. The superficial layer of protoplasm in some cases is condensed into a firmer portion continuous with subjacent less dense protoplasm. In sections such a surface condensation appears as a bright line and may extend about the entire cell, although limited in some cases to the exposed surface. Occasionally this denser layer presents perpendicular striations, which are considered as fine, hair-like processes of protoplasm held together by intervening less differentiated protoplasm. Such an arrangement is known as a striated border. The surface of other cells has a brush border in which densely packed, non-motile, hair-like, protoplasmic structures project slightly beyond the surface. The elements of such a border are associated with small granular swellings at their base and are thought to be active in absorption. The peak of such differentiation is found in the ciliated boundaries of various types of epithelial cells where the fine, hair-like, protoplasmic processes are much longer than those of the brush border. These processes, the cilia, of which there may be as many as a hundred from a single cell, often possess the power of movement. Less commonly cells have single, whip-like, protoplasmic processes, called flagella, which are usually motile.

According to some authors, cilia possess a protoplasmic shell enclosing a hollow core, which being rhythmically filled and emptied with more fluid protoplasm imparts to these structures their charac-

teristic movement. According to other investigators, cilia have a contractile band along each of two opposite surfaces and the movement is effected by the alternate contraction of these bands. In either case the movement appears as a sharp initial bending and a slow recovery. The motion of one row of cilia apparently initiates similar activity in adjacent rows, so that a series of waves pass along the field of cilia. Since the beat is in one direction, particles caught on the tips of the cilia are usually propelled in the direction of the beating. Ciliated epithelia lining the respiratory passages tend to move dust particles and material in the lumen out into the mouth or nasal sinuses. Ciliary action is independent of nerve action, as may be demonstrated by removing the ciliated membrane from the roof of the frog's mouth and studying it in a saline solution hours after the frog itself is dead. Cilia possessing the power of movement are called kinocilia, to distinguish them from static cilia or stereocilia of such cells as those lining the epididymis.

Another structural feature associated with the free boundary of some epithelia is a cuticle. The superficial protoplasm of the cell is not modified in any marked manner and is not continuous with this modified border which is formed by the cell during development. The cuticle may become impregnated with various salts and be firm and hard. The enamel of the teeth is an example of such a specialization. This type of modification plays a much wider rôle in the invertebrates where cuticles are more complex and form the skeleton of many forms.

The boundaries of epithelial cells in contact with adjacent cells are so delicate that they do not always show in preparations. Some believe a cementing substance is present between adjacent cells but it has also been indicated that the adherence of the cells to each other is due to the adhesion of adjacent cell membranes. Protoplasmic bridges may extend from cell to cell. (Fig. 16.) These have been interpreted by some as solid strands between vacuoles in the intercellular spaces or as products of technique and therefore artefacts. In silver nitrate technique silver salts are deposited about the cells so that in face view a network of polyhedral outlines define the cells as described in the case of mesothelium. The cells composing an epithelial membrane are capable of gliding by one another when the membrane is stretched.

The basal surface of simple epithelia and the basal surface of the deepest layer of the stratified types may possess a cuticle. In addition, most epithelial cells rest upon a distinct basement mem-

brane, which is a homogeneous layer of non-cellular substance derived presumably from the subjacent connective tissue with which it is most intimately associated. The basement membrane does not appear to be present in all cases. It is absent in the follicles of the thyroid gland where cuboidal cells compose the epithelial membranes.

One of the characteristics of epithelial tissues is the fact that they rest upon connective tissue. Even in those cases where the epithelial membrane is much folded, connective tissue fills in the
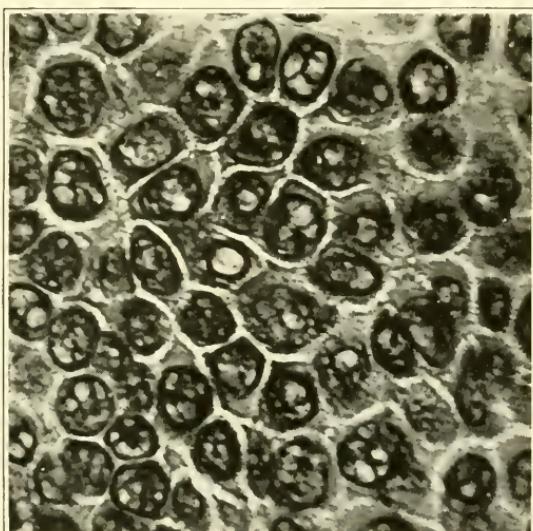
Fig. 16.—Photograph of a section through the stratified squamous epithelium of the toe of Necturus, showing intercellular strands or intercellular bridges.

spaces between the folds. This subjacent region of connective tissue carries a network of blood and lymph vessels which come into close contact with the base of every epithelial membrane, but do not extend in among its cells. There is a plexus of nerve fibers in the connective tissue below the basement membrane and some of these fibers pass along the base of the cells while others extend between the cells.

GROWTH AND REGENERATION OF EPITHELIA.

As one might expect from the exposure of various epithelia to mechanical and physiological wear, there is a varying quantitative loss of cells that must be replaced. The continual wearing away of the superficial layers is characteristic of stratified squamous

epithelia. This is especially pronounced in the case of the epidermis of the skin, which protects underlying tissues and still retains a sensitiveness to external stimuli. If the upper superficial scaly layer of dead cells continue to accumulate to considerable thicknesses, there is an increased protection with an accompanying loss of general sensitivity. This actually occurs in the callosities localized at points of constant friction and pressure. The maintenance of a protective layer of optimum thickness despite the wearing away of the superficial layers is made possible through the continual replacement of cells from the basal layers, where they are being produced through normal mitotic activity. A lesion in this type of epithelium is quickly repaired through the activity of these basal cell layers. Epithelia of other types in other locations are also subject to contact with substances causing gradual or abrupt destruction and loss of cells. In the alimentary tract, dead cells of the columnar type are replaced by mitotic activity of less differentiated cells intercalated among them. In many places mitotic activity is rarely observed under normal conditions.

In healing of surfaces from which the epithelium has been lost through injury the newly formed cells of the bordering region apparently migrate by a sliding movement to form a covering layer prior to the regeneration of the tissue characteristic of the injured region. Experiments have also shown changes in type in the case of the pseudostratified ciliated epithelium of the trachea of the cat, which, after repeated treatments with a formalin solution, became a stratified squamous membrane. In the embryo cat the esophagus is lined with a ciliated columnar epithelium, but in the adult this region has a lining of stratified squamous, the type commonly found in places subjected to external wear.

SECRETORY EPITHELIAL CELLS AND GLANDULAR ORGANIZATIONS.

Probably all epithelial cells to some extent elaborate active substances through biochemical processes of their protoplasm; these processes being collectively spoken of as secretion. Although protection and absorption are the major tasks of some epithelial cells, there are others primarily concerned with this process of secretion. In those cells active in secretion there occurs an accumulation of very small secretion granules in the cytoplasm. The origin of these granules is debated, but they appear first in the basal

region, then usually pass toward the surface of the cells where they are discharged to become part of the active secretion. There is considerable variation in the method by which secretion is carried to completion in different types of glandular cells. The use of the term glandular to indicate such secretory cells places the emphasis upon function, but the form of most of these cells will fit into the classification of types already studied.

Groups and organizations of actively secreting epithelial cells are called glands, and two broad divisions are separated on the basis of whether the secretion formed is liberated into a lumen from the free boundary of the cells or whether it passes in the opposite direction to enter the vascular system. Those organizations of cells secreting by the first method, that is by liberating their secretion into ducts which carry it to the surface of the epithelial membrane from which the gland developed, are called exocrine glands. The endocrine glands are those glandular tissues lacking excretory duct systems and whose secretions pass into the vascular system.

Exocrine Glands.—The secretory process is completed in several ways in different types of exocrine glands. In merocrine glands, granules form in the cytoplasm of the cells and accumulate toward the free boundary to be discharged as the active secretion; the process is then repeated. Most glandular secretions, such as those of the digestive glands, are derived in this manner. In a holocrine gland, as exemplified by a sebaceous gland in the skin of a mammal, the major portion of the active cell develops into a secretion mass which, when dislodged to form the secretion, is accompanied by the death and disintegration of the cell. A new cell then forms from the underlying layers of the stratified epithelium forming such glands and the process is repeated. Another type of secretion is termed apocrine, and in this case a secretion mass forms in the apical portion of the cell; this apical region becomes greatly enlarged and is finally cut off from the basal nucleated portion. This type of secretion occurs in the mammary gland. The cells whose apical portions have been cut off do not disintegrate following the liberation of the secretion but develop a new apical portion and repeat the process. A convenient classification of the exocrine glands may be built upon their organization regardless of the method of secretion.

Unicellular Glands.—In the skin of fishes and amphibians there are scattered single cells that secrete a fluid substance. Also, among the columnar cells lining the gut regions are the numerous goblet cells, already mentioned under columnar epithelia. Mucin,

which is elaborated in the cytoplasm of these cells, collects toward the free end, water is absorbed, and the volume of the secretion is increased so that the apical end becomes much distended. The distention progresses until there remains so thin a sheath of cytoplasm about the secretion that a rupture occurs and there is a discharge of the mucus. Such goblet cells apparently may undergo a number of repetitions of this apocrine type of secretion before disintegrating.

Secreting Areas.—As a further step beyond the unicellular condition, there are certain areas among cells forming an epithelial membrane where a small aggregation of cells function as a special secreting group, while the surrounding cells, although similar in form, function chiefly in protection and absorption. Such areas occur among the columnar cells lining the uterus, in the epithelial membrane lining the trachea, and in the thin membrane of cuboidal or low columnar cells forming the choroid plexes in the brain.

Glandular Pockets.—Occurring at intervals in the epithelial membranes lining the various ducts there are shallow pockets lined by secretory cells. In the trachea, cloaca, and urethra, for example, are small pockets usually lined with mucous secreting cells. Those cells near the mouth of the pocket are cuboidal or short columnar, while toward the bottom of the pocket are broader and longer columnar cells.

Simple Tubular Glands.—As the name implies, these glands result from tubular invaginations of epithelial membranes during development. The oviducts of the frog and other egg-laying forms are usually lined with tubular glands whose secretions are added to the descending eggs. The epithelial wall of the large intestine of mammals possesses a multitude of simple tubular glands closely adjoining each other, their walls composed of columnar cells, of which the majority are of the goblet type. The cavity in these tubes is called the lumen of the gland and may vary considerably in length. The secretions of the cells pass into the lumen and out to the surface of the epithelial membrane from which the gland forms. A modification of these straight tubular glands can be seen in the type represented by the sweat glands in mammalian integument. These glands have an excretory superficial portion which is long and spirals from the epidermis through the underlying connective tissue to the deeper, much-coiled portion. The deeper portion is composed of actively secreting cells and may be called the secreting end-piece of the gland. The mucosa of the fundus of the stomach

in mammals is composed of branched tubular glands with a single
short excretory duct portion and two or more longer, slightly twisted,
tubular portions composed of secretory cells. (Figs. 17 to 19.)

Simple Alveolar Glands.—These glandular invaginations develop
a spherical termination instead of the tubular type just described.
In simple alveolar glands the enlarged spherical terminal portion
is called an alveolus or acinus. These are common in the skin of
certain lower vertebrates but do not occur among the mammals.
An example of this type may be found in the skin of the frog where
a saccular base lies below the stratified squamous epithelium of
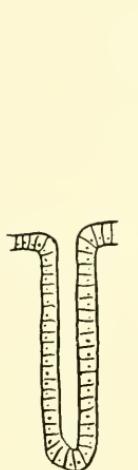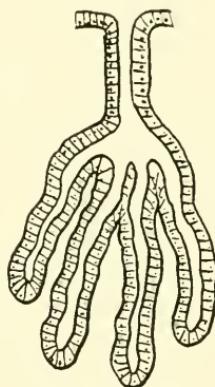
Fig. 17   Fig. 18   Fig. 19

Fig. 17.—Simple tubular gland.
Fig. 18.—Coiled tubular (sweat) gland.
Fig. 19.—Simple branched tubular gland (stomach).

the skin epidermis and connects with the surface by a passageway
through the strata of epithelial cells. This simple type of gland
may also be modified by having two or more alveoli attached to the
end of a single excretory duct, as in the case of the sebaceous glands
where alveoli are connected with the lateral walls of a common excre-
tory duct. (Figs. 20 and 21.)

Compound Tubular Glands.—The formation of compound glands
may be visualized by assuming that, instead of stopping with the
simple tubular invagination of embryonic development, the early
invagination has gone on developing branches from its deeper
portions. These secondary branches give rise to tertiary ones, and
so on. (Fig. 22.) The outermost end-portions of the smallest

branches alone possess the secretory cells and the other portions form the excretory duct system. The mucous glands of the mouth cavity, the prostate, and the kidney are examples of such tubular glands.
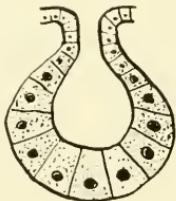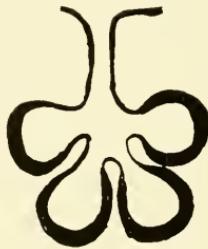
FIG. 20   FIG. 21

FIG. 20.—Simple alveolar gland (frog skin).
FIG. 21.—Simple branched alveolar gland (sebaceous).

Compound glands are invested with loosely arranged connective tissue extending between the excretory ducts and secretory end-pieces. The large masses thus separated by connective tissue are known as lobes and the smaller subdivisions are called lobules.
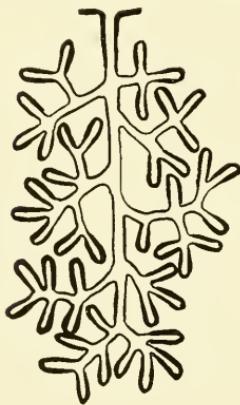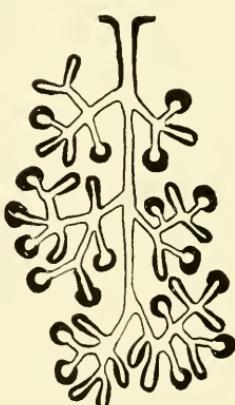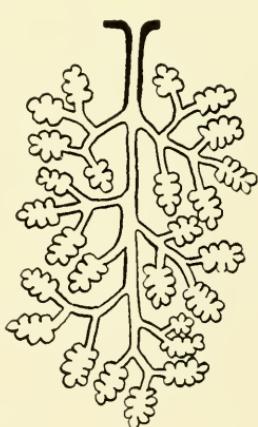
FIG. 22   FIG. 23   FIG. 24

FIG. 22.—Compound tubular gland.
FIG. 23.—Compound tubular-alveolar gland (submaxillary).
FIG. 24.—Compound alveolar gland (lung).

The main excretory ducts accompanied by blood and lymph vessels, and nerves are carried in the connective tissue between the lobules. Immediately surrounding the basement membrane of the gland cells of the secreting end-pieces is a capillary network and also a plexus of nerve fibers.

The liver in lower forms is a compound tubular gland in form, but in the higher vertebrates this form may be lost during development, as will be seen when studying this organ.

Compound Alveolar Glands.—The manner in which these glands develop is similar to that in the case of tubular glands, but the secreting end-pieces are expanded into alveoli. (Fig. 24.) The lung develops as this type of gland but is not usually considered as a gland, though it might be considered as secreting carbon dioxide and absorbing oxygen. The mammary gland is another example illustrating the structure of a compound alveolar gland. The salivary glands and the pancreas, considered as tubulo-alveolar, have the outward appearance of compound alveolar glands, but the lumens of the secreting end-pieces are tubular in form. (Fig. 23.)

Serous and Mucous Glands.—On the basis of secretory products there are some glands whose secretions are of the watery or serous type; others secrete a thicker, viscous, mucilaginous substance, called mucus. The cells secreting these two types of material have certain distinctive features. (Fig. 25.) The serous cells usually have a clouded cytoplasm with a rounded nucleus located toward the center of the cell; the mucous cells are generally larger, have a clearer cytoplasm, and have an oval, flattened nucleus located

Fig. 25.—Diagram of mucous and serous cells.

well toward the base of the cell. The secreting end-pieces of the serous glands have a small lumen when compared with the relatively wide lumen in the end-pieces of mucous glands. The pancreas presents an example of serous secretion. The salivary glands, which vary somewhat in this respect, may present either serous or mucous or a combination of both, as will be observed in studying these glands later.

Endocrine Glands.—These glandular organizations are very richly supplied with blood vessels, which their secretions enter and are circulated by means of the blood stream. The thyroid is an example of this type of gland; it begins its development as do other glands,

invaginating from an epithelial membrane, but the end-pieces become separated by the loss of the connecting duct system. As a result, the mature gland is composed of separate small spherical vesicles of varying size with their walls composed of a single layer of cuboidal epithelium. The other endocrine glands are best studied in the chapters dealing with them.

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See Appendix for general text references.

CHAPTER III.

THE CONNECTIVE TISSUES.

In contrast to epithelial tissues, where intercellular material is absent or insignificant in amount, in connective tissues the emphasis is placed upon the nature of the intercellular material. The cells of connective tissues usually form a small part of the tissue mass and are partially or completely separated from each other by the various types of intercellular material which they form. This emphasis upon the non-cellular, intercellular products of connective tissues is directly associated with the rôle they play. In general, connective tissues form the supporting structures of the body, from the heavy framework of the bony skeleton to the finer networks supporting the capillaries. In addition to the formation of intercellular products some of the cellular components of the tissues are active in storage and phagocytosis. The various types of connective tissues trace their origin back to the mesoderm of early stages of embryonic development.

Classification.—As one might expect from the nature of connective tissues, classification of the various types is based both upon the cellular constitution and the nature and arrangement of the intercellular deposits. There are intergrading forms and also some variation in the general characteristics considered as distinguishing the types, so that here, as in the case of epithelia, there may be difficulty in classification. Two types, mesenchyme and mucous tissue, are mainly embryonic. In the adult vertebrate, the following types are distinguished: reticular tissue, loosely organized fibro-elastic tissue, adipose tissue, densely organized fibrous and elastic tissue, cartilage, and bone. These types include modifications or subdivisions which may be best considered in order when describing the features of each type.

MESENCHYME.

As soon as the three germ layers are well established in the embryonic development, cells begin moving away from the mesoderm to occupy spaces between it and the ectoderm and endoderm. These early, wandering cells compose mesenchyme tissue.

They have an irregular, stellate form with branching cytoplasmic processes, and have the appearance of a syncytium. The nucleus is relatively large and mitotic figures are often evident. An apparently homogeneous, liquid, intercellular substance fills in the spaces between adjoining cells. (Fig. 26.) From such mesenchyme, or embryonic connective-tissue cells, are derived the various differentiated cells that form the cellular elements of the connective-tissue types of the adult. They also give rise to cells that in turn differentiate into endothelium, blood, and smooth muscle cells.
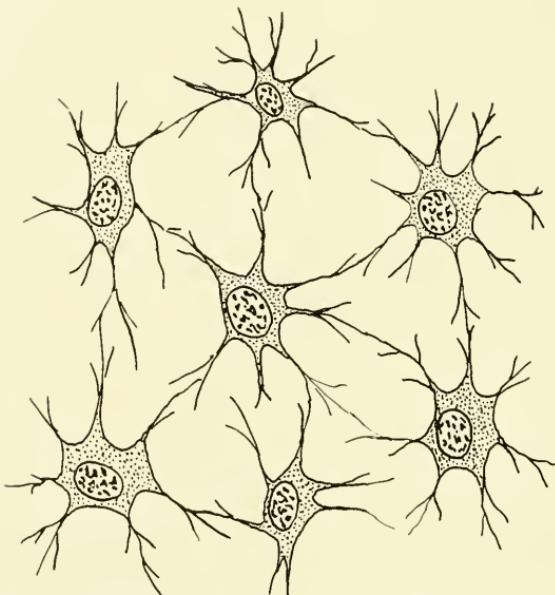
Fig. 26.—Diagram of mesenchyme cells.

MUCOUS TISSUE.

This is a special type of connective tissue found in embryos, and reacts to stains for mucus. In the fresh condition, as seen in the case of an umbilical cord, it presents a jelly-like appearance due to the abundant gelatinous intercellular material. Within this gelatinous matrix are fine fibers and scattered cells with long branching processes. The ends of the branches of one cell are in contact with other cell branches, so that a network of cells and fibers extends throughout the mucoid ground substance. In the case of the umbilical cord this tissue occurs between the blood vessels and ducts

passing from the placenta to the fetus. A similarly appearing tissue may be found in other regions of the embryo preceding the differentiation of the fibroelastic connective tissue.

RETICULAR CONNECTIVE TISSUE.

This widely distributed tissue is not easily demonstrated in routine preparations. It can be demonstrated associated with secretory epithelial organizations of glands and in the framework of lymph organs. The cells resemble mesenchyme cells but are larger, with a large, oval nucleus and long branching cytoplasmic processes making contact, if not continuing with adjoining cells. (Fig. 27.) Associated with the cells are branching fibers which have an affinity for silver salts, so that they are called argyrophil
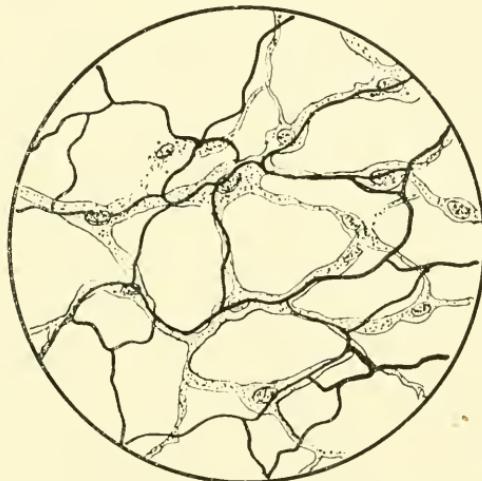
Fig. 27.—Diagram of reticular tissue.

fibers. By the use of silver salts which blacken them, these fibers can be demonstrated as branching and anastomosing to form delicate networks in all regions of the body. They are continuous with the collagenous fibers of the loose fibroelastic connective tissue which do not react in the same manner to the silver salts. Embryologically the argyrophil fibers appear before the collagenous type, so that reticular tissue may be considered as the finer, less differentiated continuations of the loose fibroelastic connective tissue, an intermediate condition between it and mesenchyme. The wide distribution of reticular tissue in close association with and transition

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to the loose fibroelastic type often make it difficult to differentiate. The appearance of the reticular cells may be studied in routine preparations of lymph glands of mammals. In the central portion of such a gland there is a diffuse arrangement of tissue and a network of reticular cells may be observed forming a support for the lymphocytes.

LOOSELY ORGANIZED FIBROELASTIC TISSUE.

This is probably the most widely distributed type of connective tissue and also contains the component elements to be found in other types. It is well exemplified by subcutaneous tissue which is easily accessible for study. When the skin is removed from a freshly killed mammal, a moist, white, filmy tissue is seen lining the undersurface of the skin. It tears easily as the skin is removed; part remains attached to the skin and part adheres to the underlying tissues of the body. It has been called areolar tissue because open spaces may be seen among the fibrous intercellular elements when spread out on a slide. In the normal state of the tissue, however, these spaces are filled with a tissue juice, or ground substance, so that it is advisable to use the longer, more descriptive term instead of the term areolar which is misleading. Study of this tissue clearly reveals cells and fibers, but the ground substance or tissue juice noted by the feeling of moisture in the fresh tissue, is difficult to demonstrate in preparations.

Cell Types.—Cells are not easily seen in fresh tissue mounts, nor is it easy to demonstrate all the types in any one preparation. They are often studied by means of intravitam staining, a process in which small amounts of harmless dyes, such as neutral red, are introduced subcutaneously in a living animal, and, later, a small amount of the subcutaneous tissue is carefully removed for study. In such cases, certain cells take up the dyes in a characteristic fashion.

Fibrocytes.—Derived directly from mesenchyme, these cells become larger, elongated, and flattened. They have long cytoplasmic processes and in edge views or sections they appear spindle- or rod-shaped. The cytoplasm is clear and stains only faintly with acid dyes, thus making the cell outline indistinct in preparations. The nucleus is relatively large and oval and appears lightly stained. A nucleolus is usually present. In some preparations furrows appear on the surface of the cells presumably as a result of their close association with fibers. Early investigators concluded that

these cells produce the material forming the fibers, and the terms
fibroblast and fibrocytes were applied to them. The long proc-
esses appear to be in contact with similar processes of neighbor-
ing cells, if not actually fusing with them, but the cells appear to
act as independent units rather than as a syncytium. It is not
believed that the fibrocytes transform into the other types of cells
found in this tissue. (Fig. 28.)

Histiocytes.—This type of cell is almost as numerous as the fibro-
cyte. It is smaller and has a few blunt cytoplasmic processes.
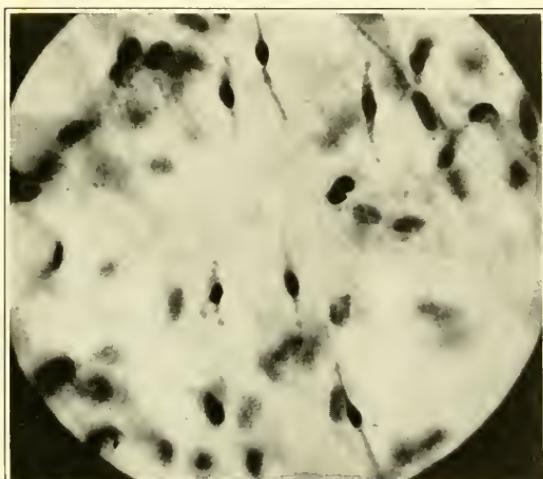
Fig. 28.—Photograph of a spread of subcutaneous tissue of the cat showing
histiocytes and nuclei of fibroblasts.

The cytoplasm stains with acid dyes, and the cellular outline is
visible. The nucleus is relatively small, oval or bean-shaped, with
distinct chromatin masse which cause it to stain darkly and stand
out clearly in preparations. A nucleolus is not usually present.
Ordinarily these cells are at rest, but under inflammatory conditions
they migrate in the tissue spaces by ameboid motion. This type of
movement is not exhibited by the fibrocytes. The histiocytes are
capable of phagocytic activity and engulf bacteria, colloidal dyes, or
other particulate matter. During active phagocytosis they are known
as macrophages and their numbers increase at points of infection and
inflammation. The terms clasmatocyte and resting wandering
cell, are also used as names for these cells.

Ameboid Wandering Cells.—These cells resemble certain of the
non-granular leukocytes of the blood so closely that some investi-

gators have concluded that many are derived from lymphocytes and monocytes escaping into the tissue from the capillaries, and others are derived from mesenchymal cells in the tissue. The nucleus is relatively large and the cytoplasm stains with basic dyes. They vary in size and shape, so that gradations from those resembling the leukocytes to those resembling typical histiocytes may be found, this being especially true in inflamed tissue.

Mast Cells.—This type of cell occurs in most vertebrates but varies in distribution. It is characterized as having cytoplasmic granules that stain selectively with basic aniline dyes. The cytoplasm of these cells is completely filled with granules, which in some animals are water-soluble. In mammals the cells are usually
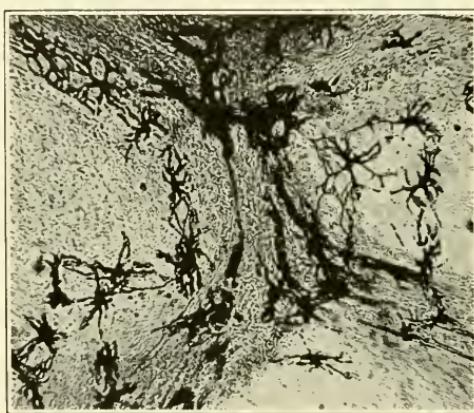
Fig. 29.—Photograph of chromatophores in the connective tissue of a section of cleared unstained lung of the bullfrog.

irregularly rounded, but in certain amphibians long cytoplasmic processes are often present. When neutral red is used as a vital stain, the cytoplasm appears filled with dark red granules, which methylene blue stains purple.

Pigment Cells.—Elongated cells with irregular cytoplasmic processes containing pigment granules occur commonly in loose fibroelastic connective tissue in certain regions and are known as chromatophores. (Fig. 29.) In mammals such pigment cells occur below the epidermis and in the choroid coat of the eye, but in lower vertebrates similar cells are much more widely distributed and occur in many internal organs. In fish and amphibians the pigment cells in the skin take part in the color changes exhibited by many forms. Such changes are considered as due to alteration in the distribution

of the granules. Pigment cells containing the black melanin granules are called melanophores; those with yellow-red granules are called xanthophores; and those with brown-red granules are designated erythrophores. No single species necessarily offers examples of all three types of cells. In some fishes guanine crystals contained in the pigment cells are responsible for the silvery color of the scales.

Undifferentiated (Mesenchymal) Cells.—Many mesenchyme cells remain undifferentiated and scattered throughout this type of connective tissue, usually near the blood vessels. Such cells are smaller than the fibrocytes but not otherwise sharply distinguishable from them in appearance. However, these cells are capable of differentiating into various types of connective tissue.

Fat Cells.—Certain cells of this tissue are characterized by a storage of fat droplets until the cell becomes distended to a spherical shape, with its nucleus and thin layer of cytoplasm pushed to the periphery. (Fig. 30.) Such cells occur singly or in groups along
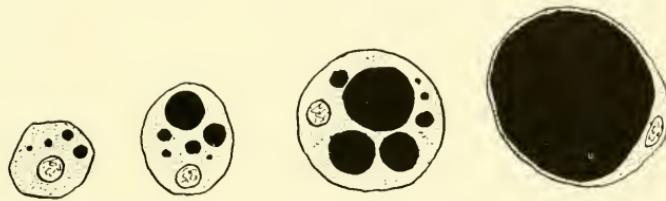
Fig. 30.—Diagram showing accumulation of fat inside of a fibroblast and the development of this into a fat cell.

capillaries, and organizations of them constitute adipose tissue, which we will consider separately.

Fibers.—The fibrous elements which give the name to this tissue are divided into two types, a white or collagenous and a yellow or elastic fiber.

Collagenous Fibers. (Fig. 31.)—These fibers are almost transparent in the living tissue and are called collagenous because they yield a gelatin when boiled in water. Fixed and stained spreads show a network of fibers extending in all directions. Study shows that the strands are bundles of microscopic fibrils, the larger strands containing more fibrils than the smaller ones. These fibrils are presumably held together in bundles by a cement substance which dissolves when fresh material is soaked in lime water. The fibrils do not branch but their organization into bundles is such that the bundles branch and form networks. In dilute alkalies like potassium hydroxide, and such acids as acetic,

the fibrils become swollen. They are digested by acid solutions of pepsin. In preparing slides to show them, fixing solutions containing acetic acid should be avoided since it causes swelling and distortion of the collagenous material. They are stained by eosin and other acid dyes but stand out sharply with aniline blue. Their properties appear to show some variation in different regions of the same animal as well as in different animals. They do not stretch, but through their arrangement in networks adapt themselves to possible expansions of the tissue by changes in the meshes.

Elastic Fibers.—Homogeneous threads or small bands of varying size occur as distinct, refractive, and non-fibrillar elements. They are composed of a compound called elastin, and branch to form a network. These fibers are decidedly elastic; under tension they stretch but resume their original form when released. When they are broken the ends curl up. Grouped in large numbers, these fibers appear yellow in color. They are not affected by boiling in weak acids, or alkalies, but are slowly digested in solutions of pepsin and trypsin. Specific stains, such as resorcin-fuchsin and orcein make them stand out as distinct branching threads in preparations. Through combination with one another these fibers form bands or membranes in the walls of large arteries.

Fig. 31.—Diagram of loose fibroelastic connective tissue. 1, bundles of collagenous fibers; 2, elastic fibers. Tissue spaces are indicated between the fibers.

Function.—Loose fibroelastic connective tissue forms a strong elastic material which is light and flexible and adapted to holding together other tissues which are especially concerned with secretion, absorption, movement, storage, and conduction. It is the chief tissue for the support of the blood vessels and the capillary networks supplying nutrition and oxygen to the tissues involved in secretion and excretion. Furthermore, before reaching other cells both food and oxygen must pass from the capillaries into the ground substance or tissue juice of this tissue. Likewise, such wastes of metabolism as urea, carbon dioxide, and water pass through the tissue juice on their way to the capillaries prior to elimination. Thus, the element

of the tissue not usually demonstrated in preparations plays an extremely vital part in metabolism. This type of tissue plays an important rôle in repair following injuries and infections and is closely linked to serological reactions which are occupying those interested in immunology.

SEROUS MEMBRANES.

The peritoneum, pleuræ, and pericardium are thin layers of fibroelastic connective tissue covered with mesothelium. The cellular elements are more numerous in these membranes than in the loose fibroelastic connective tissue of other regions. The mesenteries are composed of similar thin membranes of connective tissue with mesothelium on both surfaces. The cells of serous membranes produce a watery, somewhat viscous fluid, which lubricates the surfaces of organs as they move over one another during their physiological activities. Histiocytes as well as white blood cells, especially neutrophils, increase in number in the fluid ground substance of these membranes in inflammation. Fat cells may accumulate in certain regions to form considerable masses.

ADIPOSE TISSUE.

This is a modification of the loose fibroelastic type in which many of the cells become active in storage of fat. (Fig. 32.) The fat-storing cells are considered by some to be directly derived from fibrocytes but others believe them to be more directly derived from undifferentiated mesenchyme cells, called adipoblasts. However derived, these cells occur in large or scattered masses among the other elements of loosely organized fibroelastic tissue.

During transformation of fibrocytes or adipoblasts into typical fat cells, droplets of fat accumulate within the cytoplasm. The cell becomes distended into a spherical mass with a protoplasmic periphery. When a number of fat cells are formed close together, the spherical form of each is modified at points of contact, so that such cells appear as polyhedral bodies resembling a mass of small soap bubbles. In such groups the different portions of the cell membranes may be seen by changing focus. In a number of locations, fat cells accumulate in small or large masses, as will be observed in sections of the skin, the mesenteries, the supporting tissues of the thymus, kidney, and intestinal tract. Under condi-

tions of starvation the fat stored in these cells is liberated by metabolism and used as a source of energy. The cells then resume a condition in which only scattered droplets appear in the cytoplasm, but with favorable conditions the storage may be repeated. Fat accumulated during the summer season in hibernating animals is metabolized during the winter dormant season in maintaining the life of these forms.

In ordinary routine techniques preparing tissue for examination, the fat is dissolved out, leaving the cells as mere empty shells in the final preparation. Fat cells may be handled so that the fat is fixed, as when using osmium tetroxide, and carried through to the final preparation. Other solutions, such as Sudan III, stain fat specifically and may also be used to indicate it.
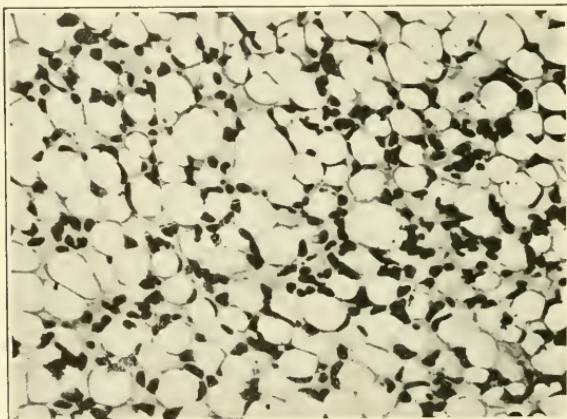
Fig. 32.—Adipose tissue of the fat body of Amblystoma.

DENSELY ORGANIZED COLLAGENOUS AND ELASTIC CONNECTIVE TISSUES.

Under this heading there are irregularly arranged tissues resembling the loose fibroelastic type but having their intercellular material present in far greater quantity. There are other types in which the intercellular fibrous materials, either elastic or collagenous, are arranged paralleling each other.

Dense irregularly arranged fibroelastic tissue with the collagenous fibers predominating and with proportionately few cells, which are mainly fibroblasts, forms the dermis of the skin in mammals, part

of the wall of the gut in all vertebrates, and portions of the wall of large bloodvessels. Its distribution and occurrence vary in different animals.

The regularly arranged dense tissue usually has either collagenous or elastic fibers predominating. In the corneum of the eye a dense collagenous tissue is present. The dermis of some animals, as exemplified by the frog, has a tissue of similar collagenous composition. A whole group of structures having a regular arrangement of the tissue elements are classified as tendons and ligaments.

Tendons.—Tendons are composed of closely packed bundles of collagenous fibrils arranged paralleling each other to form cords connecting muscles to bones. The fibrils are held together with a cementing ground substance and the entire tendon is surrounded by a sheath of loosely and irregularly arranged fibroelastic connective tissue which extends into the tendon to ensheath bundles of fibrils. A characteristic feature of tendons is the location of the cells, which are mainly fibrocytes. These cells cannot be wholly identified in sectioned preparations, but their nuclei appear as deeply stained elongated oval structures. They are located near each other in the region between adjacent bundles of fibrils. The cell form is modified by the compression of the closely packed fibrils; the cytoplasm extends out between the adjacent bundles so that each cell appears to have several flat wings. In longitudinal sections the fibrillar composition of the bundles is indicated by faint longitudinal striations. These structures supply a tough but inelastic connection primarily between the bones and muscles.

Ligaments.—Ligaments are tough fibrous bands which structurally resemble tendons except that they may be less regularly arranged and contain elastic fibers in considerable quantity. Ligaments commonly connect two bones where they form a joint. One in particular, the ligamentum nuchæ, prominent in large quadrupeds, such as cattle, is especially rich in elastic fibers. It forms a strong elastic support connecting the skull with the spinous processes of the cervical and thoracic vertebræ. It is composed of bundles of elastic fibers paralleling each other and harnessed together with loosely organized fibroelastic connective tissue. Such a structure does not fatigue easily when stretched, and is admirably suited to the demands of grazing animals.

Not all ligaments connect bone with bone. In other loca-

tions, as in the case of the uterus, a broad ligament extending from the peritoneum connects the uterus with and supports it on either side. The round or utero-ovarian ligament is a short fibrous cord extending from the uterus to the ovary on either side. Elastic cords form the ligamenta flava of the vertebræ and also the vocal cords.

CHONDROID TISSUE AND CARTILAGE.

Special dense varieties of connective tissue with cells and fibers, but with a solid substance encapsulating the cells or forming an intercellular matrix, are spoken of as chondroid tissue and cartilage.

Chondroid tissue (Fig. 33) is composed of a mass of closely packed ovoid cells, each encapsuled by a small amount of matrix. This
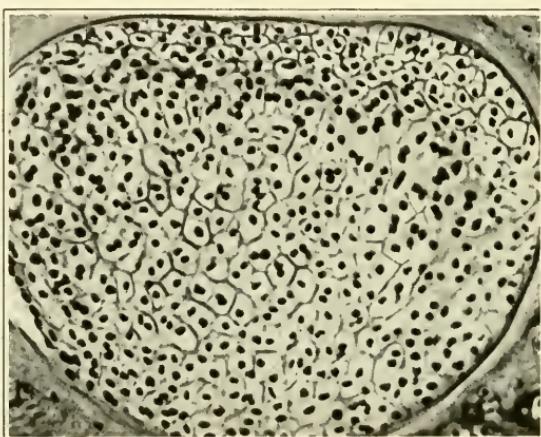
Fig. 33.—Photograph of a vertebra of a newt, showing encapsulated vesicular chondroid cells within the bony vertebral tissue.

tissue may be found associated with the tendon of Achilles of the frog. A similar tissue is an embryonic stage in the formation of cartilage in which intercellular matrix is more abundant. Where chondroid tissue will form, mesenchyme cells proliferate to form a mass of closely packed cells. These become rounded out and larger in size, and are separated by a scant amount of intercellular material in which collagenous fibrils can be demonstrated. A thin capsule of firm material staining with basic dyes forms about the cells so that a structure of considerable firmness results. Examples of chondroid tissue, or pseudocartilage as it is sometimes called, may be observed in skeletons of many fish and Amphibia as well as in the embryonic

stages of higher vertebrates. The amount of the intercellular solid matrix varies in many cases, but does not far exceed the cellular components, as in cartilage.

Cartilaginous tissue forms the entire skeleton of the elasmobranch fishes, a considerable part of the skeleton of ganoid fishes, is still a prominent element in the skeletal framework of amphibians, and, though not so important in the adult reptile, bird, and mammal, it is the material of which the embryonic skeleton of these forms is composed. Preliminary to cartilage formation, mesenchyme cells accumulate and become compactly grouped until the original stellate shape is lost. An intercellular substance, at first acidophilic and then basophilic, forms together with collagenous fibrils. To this point it resembles the chondroid tissue. By multiplication of the precartilaginous cells and increase in the intercellular material, an irregular but compact mass is formed which compresses the surrounding mesenchyme cells into the early form of perichondrium, the peripheral sheath of cells and collagenous fibers active in formation of new cartilage cells, or chondrocytes, at the periphery. The cells in the interior of the early cartilaginous mass divide, and the newly formed cells in turn secrete about themselves more intercellular matrix so that cartilaginous skeletal structures are formed. The cells are usually rounded and separated by matrix, but in some cases cytoplasmic processes may extend through the matrix to adjoining cells. On the basis of the composition of the intercellular material, three types of cartilage are commonly distinguished, namely, hyaline, elastic, and fibrous, although there are all gradations from the early chondroid or embryonic condition to any of the three named.

Hyaline Cartilage.— In living condition this widely distributed type appears clear or slightly bluish and somewhat translucent. When boiled, the matrix yields chondrin, a gelatinous material resembling the gelatin obtained when collagenous fibers are boiled. The apparently homogenous solid matrix may be dissolved by treating with trypsin and potassium permanganate and collagenous fibers become apparent. It may be regarded as a specialized form of dense fibrous connective tissue in which the matrix is a solid organization of the intercellular tissue secretions. (Fig. 35.)

Throughout the matrix cells occur in spaces termed lacunæ. These cells, the chondrocytes, are closely related to the fibrocytes. In embryonic cartilage, cells in the interior of the mass are still active in mitotic division, and when in the two-cell stage resulting

from a division, each may form matrix until they are separated into lacunæ and then divide in turn. Or a cell may divide to form two, which remain in a common lacuna, and one or both of these may divide again to form three or four. Different methods of division may occur, and result in the production of other types of cell clusters. The nuclei are rounded and relatively small. The cytoplasm appears to be rich in fluid and in the dehydration necessary for making permanent preparations it often shrinks far away from the wall of the lacuna to which it is closely applied during life. The matrix immediately surrounding the lacunæ is more basophilic than that more distant; this may be due to the absence of the acidophilic collagenous fibers in this region, or be associated with the fact that it is the most recently deposited and may differ slightly from the older material. Although embryonic cartilage increases by division of the chondrocytes within the matrix, later growth is due mainly to formation of new cells and cartilage on the periphery through the activity of the inner zone of the perichondrium. The cells along the inner surface of this sheath divide and differentiate to form young cartilage cells, which in turn form matrix which separates them from the sheath and adds to the cartilage mass. Blood vessels and nerves extend into the outer portion of the perichondrium but not into the matrix, so that nutritive materials received by the central cells must reach them by diffusion through the matrix.

Cartilage proper usually is markedly basophilic, but perichondrium takes acid stains. The intermediate region of developing cartilage about the periphery shows a transition from the acid-staining condition of the perichondrium to the basic staining matrix of the inner region of the cartilage, indicating a chemical change during development. Cartilage present in some elasmobranchs becomes calcified and forms a much harder and more rigid support than the commoner hyaline cartilage.

Elastic Cartilage.—In the fresh condition this type has a yellow tinge due to the prominence of the elastic fibers in the intercellular material. There is relatively little matrix aside from some forming thin capsules about the cartilage cells. In this type the elastic fibers form a coarse network continuing with the perichondrium. Elastic cartilage occurs in the external ear of mammals and in the smaller bronchi.

Fibrous Cartilage.—In this type there is an abundance of collagenous fibers which form a network about the cartilage cells.

Very little basophilic matrix is formed and it appears intermediate between the dense fibrous connective tissue of perichondrium and hyaline cartilage; the intercellular material resembles that in perichondrium or tendons, but the cells are ovoid and characteristic of cartilage. It occurs, for example, covering the surfaces of vertebræ where they make contact.

BONE.

This is the last type of connective tissue to make its appearance during embryonic development and is also the hardest tissue. The skeleton of the majority of the higher vertebrates is first laid down as a cartilage but is later replaced by bone, although some bones arise directly from differentiation of mesenchyme cells of the embryo. In each case the nature of the bone formed is the same but the processes involved are separated into a direct or intra-membranous ossification, and an indirect or endochondral ossification in which the bone is preceded by a cartilage mass. The special-ized connective-tissue cells through whose activity the matrix of bone is formed are called osteoblasts. As a result of intercellular deposits these cells become embedded in matrix and occupy spaces, or lacunæ, in the matrix. Processes of their cytoplasm extend in small channels, or canaliculi, to make contact with adjacent cells.

Bones are composed of about 30 per cent organic and 70 per cent inorganic material. The inorganic material consists chiefly of calcium phosphate, with small amounts of calcium carbonate, mag-nesium phosphate, and sodium chloride. When fresh bones are boiled in water, a gelatinous organic substance called ostein is obtained, and is similar to the collagen derived from other types of connective tissues. If fresh bones are placed for a long period in a weak solution of nitric or hydrochloric acid, the inorganic material is removed and a flexible decalcified bone is left. Heating bone to red heat destroys the organic matter and leaves a hard, brittle substance. Structurally, bone consists of small bundles of collagen-ous fibers impregnated with calcium salts.

Intramembranous Ossification.—The flat bones of the cranium and face develop directly from sheets of mesenchyme tissue in which ossification begins at one or more central points. At these points, mesenchyme cells differentiate into osteoblasts which deposit acidophilic, fibrillar, intercellular substance. Calcium salts are deposited in this matrix which increases in amount to form spicules

of matrix that unite to form trabeculæ radiating in all directions. (Fig. 34.) The osteoblasts surrounding such spicules resemble an epithelial membrane and their continued activity results in growth of the spicules in thickness and length. With increasing deposits of matrix, some of the osteoblasts become surrounded and remain in lacunæ, in which location they are called osteocytes. Processes of these imprisoned cells radiate through canaliculi in the matrix to make contact with cytoplasmic processes of adjoining cells. After the appearance and early activity of these ossification centers, the mesenchyme surrounding the developing spongy plate condenses
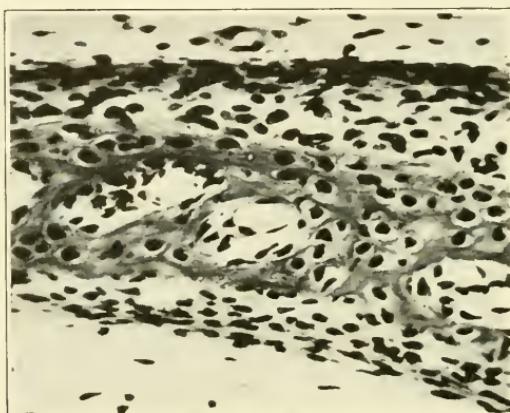
Fig. 34.—Photograph of a developing membranous bone in the head of an embryo cat. The mesenchyme cells are concentrated in the peripheral region to be occupied by the periosteum. A number of cells are imprisoned in an acidophilic fibrous matrix in which calcium salts are depositing. In the 3 small central spaces primary marrow cavities are forming and contain blood elements and osteoblasts.

into a fibrous membrane, the periosteum. From its inner surface osteoblasts are differentiated and through their activity parallel plates, or lamellæ, of compact bone are formed, a process known as periosteal ossification. Blood vessels forming in the connective tissue between the spicules of bone connect with vessels developing in the periosteum, and other mesenchyme cells in these regions among the spicules give rise to reticular tissue, adipose cells, and developing blood cells, which together represent the embryonic bone-marrow.

As a result of ossification to this point, upper and lower, or inner and outer, plates of compact bone are connected by a central region of spongy bone. This early bone is not permanent, for a moulding of the mature bone involves resorption of much of the first bone

formed. During the resorption giant multinucleated cells called osteoclasts appear along the lamellæ undergoing resorption. To them has been attributed a part in the process of dissolution without other evidence than their presence in such a location. The flat bones grow until they meet adjacent plates, at which time a fusing of their interlacing edges follows and growth ceases.

Endochondral Ossification.— In this type of bone formation a hyaline cartilage forerunner roughly outlines the bone which is to replace it and after its removal the resultant bone formation is fundamentally the same as in intramembranous ossification. In the center of the cartilaginous piece ossification activity is preceded by enlargement of the cartilage cells and their arrangement into rows accompanied by a calcification of the hyaline matrix separating them. Two things happen simultaneously. From the inner cellular region of the perichondrium, bud-like vascular tufts burrow into the cartilage and develop a primary marrow tissue. Within the center of the cartilage a number of cartilage cells and some of the matrix disintegrate and are resorbed, and into the spaces thus formed the tufts of primary marrow tissue extend. These invaginations of tissue carry in osteoblasts and other elements of bone-marrow, which continue to extend into the progressively forming cavities. At first, the osteoblasts deposit bony matrix on calcified cartilage spicules not resorbed in the first dissolution process and then at many other points form spicules of bone in the same manner as in intramembranous bone formation. (Fig. 35.) The dissolution of cartilage progresses toward either end of the cartilage piece and marrow tissue deposits bony spicules until the major portion of the cartilage region has been replaced by spongy bone. Associated with the destruction of the cartilage are chondroclasts, large multinucleated cells.

With the initiation of ossification within the cartilaginous piece, there also occurs a change along the periphery where the inner cells of the perichondrium become osteogenic, and this outer fibrous sheath surrounding the developing bone becomes the periosteum. In the same manner as in the plates of membranous bone, osteoblasts from the periosteum then deposit peripheral or periosteal lamellæ about the spongy bone developing internally.

In this type of bone there is also a resorption of the early bony matrix in the course of growth and moulding of the mature bone. During these disintegration processes, tubular channels become hollowed out and the osteogenic tissue projecting into them deposits

secondary bony matrix in the form of concentric lamellæ, one within the other, surrounding a central cylindrical cavity containing some osteogenic tissue, blood vessels, and nerve fibers. Such a series of lamellæ with the cavity just described is called an Haversian system; the lamellæ are Haversian lamellæ and the central cavity is
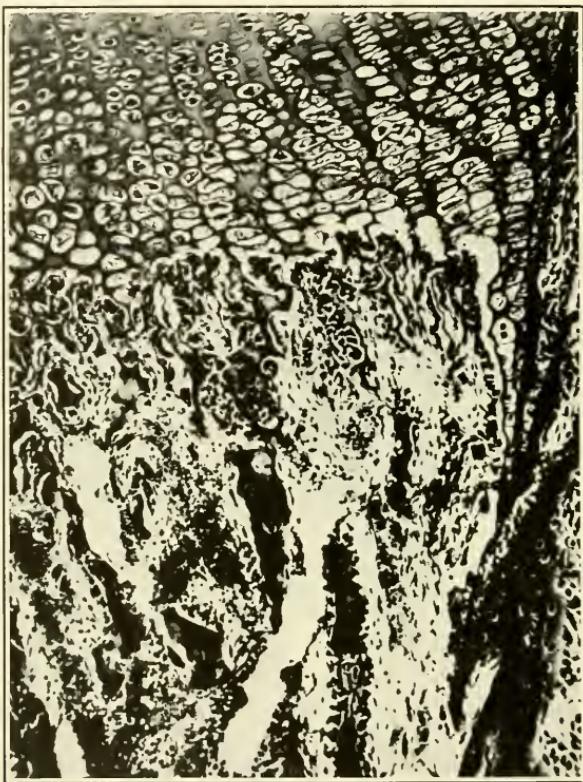
FIG. 35.—Photograph of a portion of the diaphysis shown in Fig. 36. The arrangement of the cartilage cells, the resorption of the matrix between the adjacent cells and the extension of marrow tissue are shown in studying from top toward the bottom. In the central and lower portion bony spicules have been deposited by the osteoblasts; at the right (dark portion) the periosteum is depositing layers of bony matrix in the same manner as in the case of membranous bone. A projection of vascular tissue (light area beginning in middle of right margin) is shown extending from the perichondrium through the periosteal bone into the central marrow tissue.

the Haversian canal. These systems run primarily lengthwise of the bone, but there are lateral connections between adjacent Haversian canals. Many small canals without lamellæ extend from the surface of the bone into Haversian canals and similar canals extend from the central marrow cavity outward connecting with Haversian

canals. These are called Volkmann's canals and carry vascular tissue into the Haversian canals. In many places bundles of collagenous fibers from the periosteum extend into the periosteal bone to form Sharpey's fibers which tightly attach the periosteum to the underlying bone.
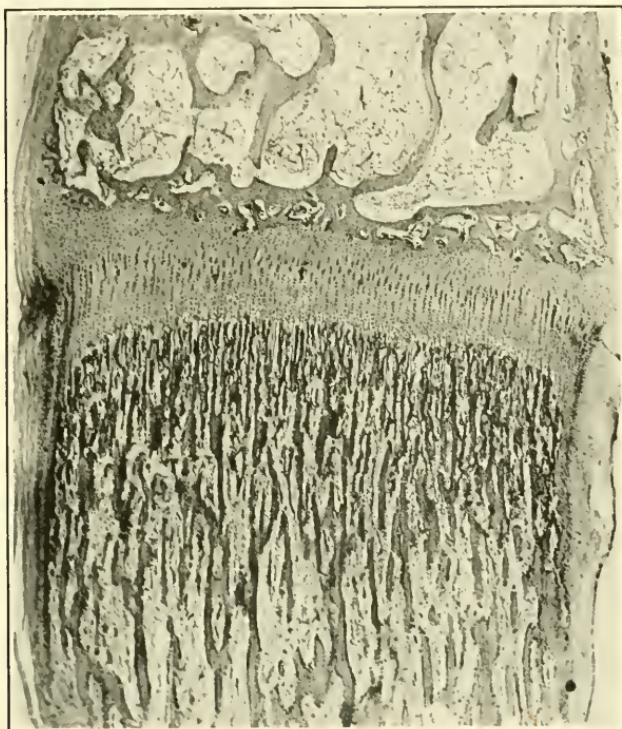
FIG. 36.— Photograph of a longitudinal section of the developing rabbit femur. Part of its diaphysis (below) and an epiphysis (above), show endochondral bone formation. In both, the cartilage is resorbed and replaced by spongy bone, in the epiphysis the final bone is irregular and spongy, but in the diaphysis Haversian systems are formed. Growth of the intervening hyaline cartilage region and progressive resorption of it and replacement by bone make possible growth in length of the bone. In the epiphysis there is a network of broad bony pieces and large marrow cavities; in the diaphysis there are more numerous thinner bony spicules separated by narrow cavities. The majority of the bony strands here are deposited paralleling the length of the bone along lines indicated by the paralleled columns of cartilage cells which are resorbed as the marrow tissue extends. Fig. 35 shows the right portion of the section in greater detail.

Growth in thickness of a long bone is made possible by resorption; this increases the central marrow cavity, and new bone is added as periosteal lamellæ. Resorption extends into primary Haversian systems, carrying along osteogenic tissue into the marrow cavities

5

so formed, and new Haversian systems are formed. The central marrow cavity becomes lined with endosteum which resembles periosteum and forms endosteal lamellæ.

Growth in length of long bones is brought about by the continued formation of more cartilage at the extremities of the shaft and its progressive replacement by bone. (Fig. 36.) Between birth and maturity ossification centers appear in the terminal cartilage pieces or epiphyses and form spongy bone similar to that formed in the vertebræ. In both of these locations Haversian systems are not formed. Each bony epiphysis is separated for some time from the end of the shaft or diaphysis adjacent to it by a plate of cartilage, but at maturity even these plates change to bone so that one continuous bone is formed and growth in length is at an end. The ends of bones retain a disc of cartilage which acts as a pad in the region of joints.

In the case of endochondral bone formation in the small lower vertebrates, the process of bone deposition appears to be primarily peripheral, little spongy bone being formed in the course of cartilage resorption of the shaft.
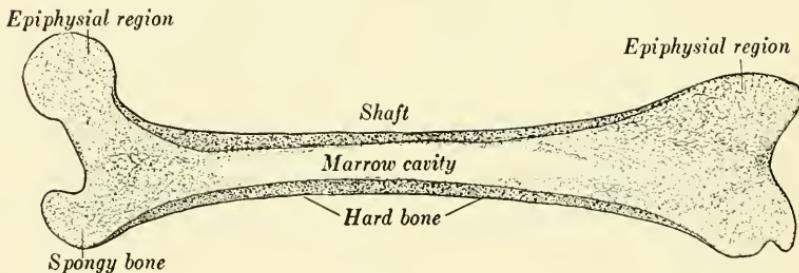
Fig. 37.—Diagram of a long bone.

Microanatomy of Long Bones. (Fig. 37.)—Let us take a long bone such as the femur and divide a consideration of it into the following parts periosteum, epiphyses, diaphysis, and the marrow.

Periosteum covers the bone except at the end-surfaces exposed to contact with adjacent bones at the joints where perichondrium and a pad of cartilage occur. Periosteum is a dense, fibrous connective tissue externally but grades into material of looser texture toward the bone. It supports arteries, veins, lymphatics, and nerves, which are carried into the Haversian canals of the bone by Volkmann's canals. The bundles of collagenous fibers, Sharpey's

fibers, extending from the periosteum into the bony matrix hold
the two tightly together.

Each epiphysis is larger than the shaft in diameter and has a
very thin peripheral layer of hard, compact bone enclosing a mass
of spongy bone between whose spicules is red marrow. Among
the constituents of red marrow are: a small amount of loose fibro-
elastic connective tissue and some fat cells; reticular tissue; erythro-
cytes; and a variety of cells in different phases of differentiation from
primitive blood cells to both red and white blood cells; blood vessels;
lymphatics; and nerve fibers.
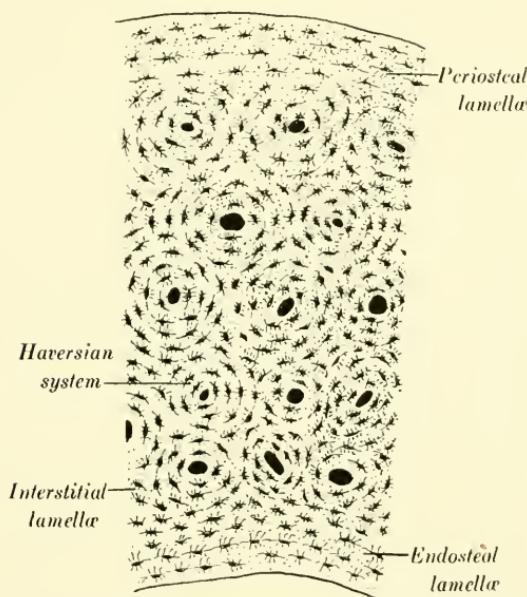
Fig. 38.—Diagram of cross-section of shaft of a long bone.

The diaphysis has four systems of lamellæ. (Fig. 38.) Imme-
diately below the periosteum are several periosteal lamellæ encircling
the shaft, these are called the external circumferential lamellæ.
Internally, adjacent to the marrow cavity, a few similarly disposed
endosteal lamellæ occur and are described as internal circumferential
lamellæ. Between these outer and inner lamellæ are arranged sys-
tems of Haversian lamellæ. Portions of Haversian lamellæ left
over from earlier Haversian systems are called interstitial lamellæ
and occur between complete later Haversian systems. Attention has
already been called to the lacunæ and canaliculi occupied by the

osteocytes in living bone. (Fig. 39.) These cells secure their
nutrition from the Haversian canal which carries the vascular tissue.
There is one main nutrient foramen near the middle of the shaft
which carries the medullary artery from the periosteum into the
central marrow cavity and at the epiphyses there are several
smaller foramina. Within the matrix of the bone there are numerous
collagenous fibers extending in different directions in adjacent
lamellæ, thereby increasing the strength of the shaft. In the central
marrow cavity the same marrow elements are present as in the
epiphyses but there is an increasing accumulation of fat cells in
this tissue with advancing age and loss of blood-forming activity.

Fig. 39.—Photograph of a portion of a cross-section of an Haversian system
showing the lacunæ and canaliculi occupied by the osteocytes in living bone. The
alternating dark and light lamellæ indicate differences in their fibrous organization.

Marrow.—In young animals, medullary spaces in bone are filled
with red marrow. This is composed of very loose fibroelastic con-
nective tissue cells, reticular fibers and cells, nerve fibers, a few fat
cells, medullary sinusoids, a great variety of primitive blood cells
and stages in blood cell formation, mature blood cells, and mega-
karyocytes. Since it is an hemapoietic center it is more fully ex-
plained under blood.

With increasing age the marrow in the medullary cavity of the
shaft changes in character. The hemapoietic property is lost. A
great predominance of fat cells develops and since it has a light
yellow color it is known as yellow marrow. The marrow in the
epiphyses remains red.

THE NOTOCHORD.

A rod of cells, the notochord, forms just dorsal to the primitive gut during the embryonic development of all vertebrates. These cells become distended to a spherical form by a considerable content of fluid and by their turgidity may give some support to the early embryo. The nuclei are small and centrally located. The position of the nuclei and the spherical shape of these cells help to distinguish them from fat cells, which they resemble in routine preparations. This tissue is supplanted by cartilaginous tissue forming from mesenchyme in the surrounding region.

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See Appendix for general text references.

CHAPTER IV.

THE BLOOD.

BLOOD might well be considered under connective tissue as a type in which the intercellular material, the plasma, is a fluid carrying a variety of free cells. Both the fluid and the cellular elements circulate through the body in endothelial-lined vessels and act as media for metabolic exchanges. The blood also plays a vital part in integrating and regulating the activities of the other tissues through the endocrine secretions which it carries.

THE PLASMA.

Although the plasma ordinarily presents no structural features in histological preparations, its physiological importance should not be overlooked. Biochemical and biophysical studies show the plasma to be composed largely of water with numerous substances in solution. Various salts, sugar, proteins, and fat are held at relatively constant concentration levels in the blood. During circulation they are made available to the various cells of the body by diffusion through the capillary walls into the tissue juice at the same time that metabolic wastes are entering the blood through the capillaries from the tissue juice. The balance is kept constant by a continuous replenishing of the various nutritive substances coupled with an equivalent removal of wastes. Since some knowledge of the rôle of the plasma in relation to processes concerned with the internal environment of the organism is essential to an understanding of the functioning of the tissues and organs, the student is advised to consult the references given at the end of the chapter.

THE BLOOD CELLS.

The cells of the blood are divided into two main groups: the the erythrocytes, or red blood cells, and the leukocytes, or white blood cells. In addition to these there may be small cytoplasmic bodies, the platelets in the case of mammals, and spindle cells or thrombocytes in lower forms.

Erythrocytes.—These cells take their name from the red color they give the blood *en masse*, but single cells have a pale yellow-green color. In mammals the erythrocytes are enucleated, that is they lose their nuclei before entering the circulating blood, but in other vertebrates they are true cells, usually flattened ovals in shape, with a central nucleus causing a cellular bulge. There is great variation in their size in different species, as evidenced by the following list of measurements: in Amphiuma (Fig. 40), which has the largest, the cells are about 80 microns in diameter; in the frog they are 22.3 by 15.7 microns; in the lizard they are 15.8 by 9.9 microns; in the rabbit the diameter is about 6.9 microns; in the cat, 6.3 microns; in the musk deer, 2.5 microns; and in man, 7.5
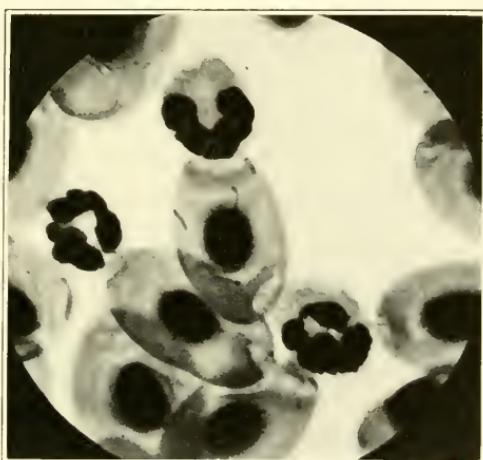
Fig. 40.—Photograph of blood cells of Amphiuma, showing erythrocytes and heterophils.

microns. In many species, especially those with nucleated erythrocytes, there may be a considerable variation in size in the same individual.

When a drop of fresh mammalian blood is spread out in a thin film on a glass slide, the erythrocytes tend to collect in groups called rouleaux, strings of bi-concave discs. The formation of such strings indicates a certain cohesiveness in the limiting membranes. Two components of these cells have been distinguished, a stroma or framework which is an optically homogeneous colloidal substance, and hemoglobin which is a conjugated protein composed of globin and a colored compound of iron called hemochromogen. The

hemoglobin is discharged from cells subjected to various agents in the process known as hemolysis. It can be induced experimentally by increasing the concentration of salts in the fluid surrounding the cells, by repeated freezing and thawing of blood, by addition of chloroform or ether, and by the action of electric currents. Hemolyzed blood cells are said to be laked, and when this occurs in the circulating blood, as it does in the case of certain pathologic conditions, the hemoglobin is eliminated from the plasma in the kidneys and passes out with the urine.

The surface of the red cell is somewhat more dense than the interior protoplasm. It is semipermeable and in osmotic equilibrium with the solutions normally present outside and is said to be isotonic with them. Mammalian plasma has about the same osmotic pressure as an 0.85 per cent sodium chloride solution, which is isotonic with the protoplasm within the red corpuscle. Frog plasma is similar to a 0.7 per cent sodium chloride solution. If distilled water is added to a drop of fresh blood on a slide, microscopic observations show the erythrocytes increasing in size as they absorb water. If the swelling is allowed to continue the cells become spherical, and hemolysis occurs. This change is a result of endosmosis, since the solution has a concentration less than that of the protoplasm within the red cells. Such a solution is said to be hypotonic. A reversal of this process occurs if the external medium is more concentrated, or hypertonic. Water is then extracted from the red cells which become wrinkled or crenated, and finally hemolysis takes place.

Oxygen diffuses through the membranes of the respiratory surfaces into the tissue juice and then through the endothelial walls of the capillaries. As the erythrocytes circulate slowly through the capillaries investing the air sacs of the lungs, or through the capillaries in the gills and skin of aquatic animals, the hemoglobin unites in a weak chemical combination with oxygen to form oxyhemoglobin. In this form oxygen is carried to the various tissues of the body and is given up as needed. The surface area of all the red cells may be computed on the basis of individual cell size and the estimated number of red cells in the body. In the case of man, estimates from 2500 to 4500 square meters have been worked out, areas many times that of the body. Depending largely upon the severity of service, there is a limit to the functioning of the red cells. When the colloidal composition ages beyond a certain point a granular condition results and the red cells are broken up. These

cells are removed from the circulation in the spleen and liver and new cells arising in the blood-forming centers take their places.

The reasons given for the segregation of hemoglobin in special cells or corpuscles, a condition characteristic of vertebrates and rarely found in animals of the lower phyla, is that capillary walls are so constructed that if the hemoglobin were free in the plasma it would leave the capillaries and be eliminated from the circulation, as occurs in cases where red cells are destroyed, as in malaria. Further-more, the retention of hemoglobin within the capillaries, should it be free in the plasma, would necessitate the capillary walls being so constructed as to prevent its passage. If this were so, various

Erythrocyte   *Lymphocytes*   *Monocyte*

Neutrophil   *Eosinophil*   *Basophil*
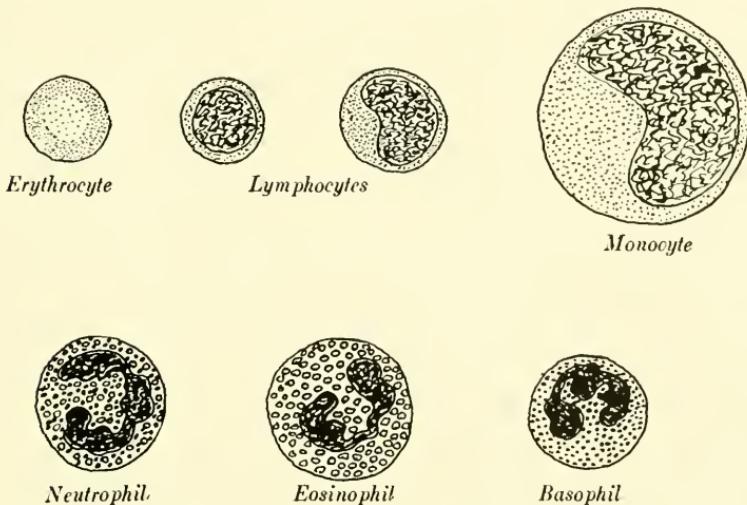
Fig. 41.—Diagram showing types of leukocytes. The size is shown in comparison with that of an erythrocyte.

other substances whose passage through such walls is essential would also be retained. In its present arrangement the hemoglobin held in the red cells is surrounded by a fluid stroma whose constantly balanced properties permit it more efficient functioning than would be possible if it were free in the plasma of the blood, where the sodium chloride concentration would interfere with its function.

Leukocytes.—These elements of the blood are called white blood cells and all are nucleated. Special stains make possible a differ-entiation of five distinct types, which can be separated into two classes. One class lacks granules in the cytoplasm and its cells are known as agranulocytes; the other class includes those cells with a distinctly granular cytoplasm, the granulocytes. (Fig. 41.)

AGRANULOCYTES.—Two types of cells are differentiated in this class, lymphocytes and monocytes, although there are numerous apparently transitional forms and variations.

Lymphocytes.—Lymphocytes are small cells about as large as the red cells in mammals, but smaller than the erythrocytes in many lower vertebrates. The cell body is round in form, with a relatively large spherical nucleus and only a thin rim of basophilic cytoplasm. The nucleus stains darkly, showing a chromatin network and usually a nucleolus. The cytoplasm takes a light blue tint with the usual blood stain (Wright's) made from methylene blue and eosin.

Variations in the size of lymphocytes are common and three sizes, large, medium, and small, are often designated. The small lymphocytes are usually meant when lymphocytes are mentioned without any qualification as to size. The medium and large lymphocytes are limited to the lymph glands and bone-marrow, occurring but rarely in the circulating blood under normal conditions. The lymphocytes cannot be looked upon as fully differentiated cells, for they are believed to have the ability to develop not only into lymphocytes of various sizes but into monocytes and indirectly into fibroblasts and histiocytes (macrophages). Lymphocytes leave the capillaries by ameboid motion to invade the tissues, so that they are mainly extravascular elements. Their invasions often carry them into the lumen of the alimentary tract and great numbers are lost in this manner. They have a slight phagocytic action and are found invading all tissues under conditions usually associated with inflammation. Nothing very definite can be said of their rôle as lymphocytes, although as wandering elements capable of differentiation into other cell types their rôle is important. Their source is mainly the lymph glands and the spleen in mammals, but in lower forms they may have their origin in the same centers as the other blood cells.

Monocytes.—Monocytes are larger than lymphocytes, with a spherical or indented, more lightly staining nucleus located eccentrically in the basophilic cytoplasm. These cells have a marked motility intravascularly and are actively phagocytic for foreign materials, including cell débris and bacteria. They are believed by some investigators to transform into macrophages when becoming actively phagocytic, and into fibroblasts when they pass from the capillaries into surrounding connective tissues. Many of the features of enlarged monocytes, macrophages, and fibroblasts make clear differentiations of types impossible. Various gradations of cells

from typical lymphocytes to monocytes are found, indicating their source as the lymphocyte.

Under conditions of chronic inflammation, cells derived from lymphocytes are present. These cells, called plasma cells, have an eccentric nucleus in which the chromatin material radiates from the center like the spokes of a wheel.

GRANULOCYTES.—This group of cells shows a more restricted development. The various types of cells constituting it are differentiated as end-products and do not possess the ability to multiply or give rise to other cell types, as in the case of the agranulocytes. They are formed in the bone-marrow or in other hemapoietic centers and are all about the same size. On the basis of the reaction of the cytoplasmic granules to certain stains three types of granulocytes are differentiated, namely, neutrophils, eosinophils, and basophils.

Neutrophils (Heterophils).—Among the mammals these cells are a little larger than the red cells, while in the lower forms they may be smaller. The cytoplasm is filled with numerous uniformly small granules. In man they are neutrophilic, staining a lavender color, but in lower forms they may take either acid or basic, or both acid and basic dyes. The nucleus is polymorphic, with several small deeply staining lobes connected by thin strands of nuclear material. Neutrophils are the most motile of the granulocytes. They are markedly phagocytic for many bacteria, and when thus actively engaged are called microphages. Great numbers are found in areas of infection and numerous dead neutrophils appear in pus, though they do not normally occur extravascularly. They are formed mainly in the bone-marrow or in other hemapoietic centers.

Eosinophils.—These cells have less polymorphic or even spherical nuclei that are lightly staining. The cytoplasm contains numerous uniformly large acidophilic granules. Eosinophils are less motile than neutrophils, have but a slight phagocytic action for bacteria, and are often found extravascularly. They are found in increased numbers in cases of parasitism by worms and under other circumstances that have led to the conclusion that they have a rôle in detoxification and in allergic conditions. In some forms, such as cats, rats and mice, eosinophils appear to be absent.

Basophils.—In these cells the nucleus is slightly lobed or spherical, it stains faintly, and is located centrally. The cytoplasm contains large variable basophilic granules, usually less numerous than those of either neutrophils or eosinophils. This type of cell is extremely

rare in man, but occurs in greater numbers in lower vertebrates. It has less motility than either of the other types of granulocytes and little is known concerning its function. The invasion of foreign materials may produce an increase in their number. They closely resemble the mast cells found in loose fibroelastic connective tissues.

Platelets.—In addition to the red and white cells of the blood there are found in mammals certain cytoplasmic elements called platelets. These are much smaller than the red cells and in smears take the blue basic dye and appear in clumps or masses. They are thought to be associated with blood clotting, though not found in clots. Their origin is traced to large giant cells in the bone-marrow, known as megakaryocytes, which are thought to extend pseudopodia into the marrow sinuses where they are cut off as platelets.

Thrombocytes or Spindle Cells.—In the blood of vertebrates below mammals platelets are not usually found, but a cellular element, the spindle cell or thrombocyte, is thought to have an analogous rôle in blood clotting. These cells are about the size of the red cells or lymphocytes and have a heavily staining spherical or oval nucleus in which a nucleolus is usually not present. The cytoplasm is clear and varies in its reaction from a more or less neutral to a lightly basophilic condition. The cells appear oval or spindle-shaped in outline and several cells may fuse and give the appearance of a cytoplasmic mass with several nuclei. The origin of these cells is traced to differentiation of the endothelial cells and of cells indistinguishable from lymphocytes.

Blood Cell Formation. Hemapoiesis is the name given to the process of blood cell formation, and a study of it in different groups of vertebrates shows some variation in its location. Embryologically blood cells as well as the blood vessels originate in mesenchyme, occurring first as isolated masses or cords, called blood islands, in the wall of the yolk sac. The peripheral cells of these masses become flattened and form the endothelium of the vessels, while the central cells become surrounded by plasma and form the first blood cells. Other similar centers arise from the mesenchyme of the embryo proper and with subsequent development a vascular network is formed that gradually gives rise to a system of blood vessels and developing blood cells. During the early stages blood cells are formed by division of the primitive blood cells in the vessels and to a lesser extent from the surrounding endothelial cells. After this early embryonic stage, blood cells no longer proliferate in the vessels, but

certain centers are developed from mesenchyme in different regions of the body. A study of the vertebrates, beginning at the lowest end of the scale with the fish and progressing through to the mammals, presents a series in which evolution of blood-forming centers may be traced and compared with similar stages in the embryonic development of mammals.

The earliest hemapoietic center to appear is found in the case of the hagfish, where a diffuse arrangement of proliferating and differentiating blood cells occurs in the connective tissue of the gastrointestinal tract. In other forms, blood-forming tissue becomes more localized and concentrated in certain regions of the tract, as in the spiral valve of the lamprey. A still further condensation gives a spleen that is bound into the wall of the stomach or intestine. Such centers are supported by a network of connective tissue in which the blood cells are proliferating in close contact with sinusoidal capillaries leading into the venous system. In ganoids the spleen is an extra-enteral organ attached to the mesentery; the submucosa of the intestinal tract may still retain the capacity to develop granulocytes, but red cell formation centers in the spleen. In the higher fishes and in the amphibia, the spleen becomes the main center for blood cell formation. Mesenchyme cells in other localities, such as the capsule of the gonads and in the liver, also play a rôle in blood cell formation. With the development of hollow bones in reptiles, birds, and mammals, the center for production of the red cells and granulocytes shifts to the bone-marrow, the spleen playing its major rôle only in early development. With this shift to the bone-marrow the production of agranulocytes, primarily lymphocytes, is taken care of by lymphoid tissue in the form of lymph nodes and the spleen.

Except in embryonic life, where blood cells form in the developing vessels, blood cell formation usually takes place extravascularly. In the hemapoietic centers there is a network of reticular cells and fibers supporting the proliferating primitive blood and lymph cells derived from mesenchyme. Following a period of differentiation into the various types of cells the fully differentiated red or white cells enter the venous sinuses which form a network through such centers. In mammals, where lymphocyte production does not commonly occur together with that of the other myeloid elements, which are primarily produced in the marrow, the lymphocytes enter lymph vessels supplying the lymphoid tissue, or, as in the spleen, enter

sinuses collecting into veins. In the case of bone-marrow a number of types are found, grading from the stem cells, or hemocytoblasts, to the fully differentiated granulocytes and erythrocytes.

Bone-marrow.—As already noted in the study of bone, there are two types of marrow, red and yellow. Yellow marrow is composed mainly of fat cells and is located in the medullary canal of the shaft of long bones, but red marrow with few fat cells and many blood-forming cells is located in the spaces between spicules of spongy bone. Such places occur in the epiphyses of long bones, in cranial bones, ribs, and the sternum. These locations are well protected, richly vascular, have a low blood-pressure and a slow circulation apparently essential for blood cell formation. There are two main constituents of red marrow, the stroma and free cells. The stroma consists of a network of argyrophil fibers and reticular cells and a small amount of fibroelastic connective tissue supporting small arteries, veins, lymphatics, and nerves. The capillaries are sinusoidal in character, and fully developed erythrocytes and granulocytes formed in the intersinusoidal tissues make their way through them into the general circulation. In the stroma are cells in various stages of differentiation, ranging from stem cells to fully differentiated erythrocytes and granulocytes. (Fig. 42.)

Hemocytoblasts.—These are generalized cells similar to large lymphocytes and are the stem cells from which erythrocytes and granulocytes develop. They may have an ameboid appearance. Their cytoplasm is non-granular and basophilic; the nucleus is large and oval with a coarse chromatin network.

Erythrocyte Formation.—Certain hemocytoblasts differentiate into round erythrocytoblasts with a spherical nucleus, and following a series of mitoses accompanied by increasing differentiation they become erythrocytes. In the erythrocytoblast stage the cytoplasm reacts to both acid and basic dyes. As the hemoglobin increases the basophilic reaction diminishes, and in the normoblast stage the cells have considerable hemoglobin in the cytoplasm and the nucleus is relatively smaller. These changes become more prominent with further mitoses, until mitotic activity comes to an end. In mammals the nucleus is extruded from the cell body, which then passes into a sinusoid and so into the vascular system. Series of stages from hemocytoblasts to newly formed erythrocytes may be found in red marrow.

Granulocyte Formation.—Certain hemocytoblasts undergo mitoses during which a differentiation of another kind takes place and gives rise to one or another of the three types of granulocytes. An early

stage is recognized as the premyelocyte, of which three forms can be distinguished on the basis of cytoplasmic granulation. The nuclei are spherical or reniform at first, but each type undergoes further mitoses with further differential granulation of the cytoplasm and lobulation of the nucleus until neutrophil myelocytes,
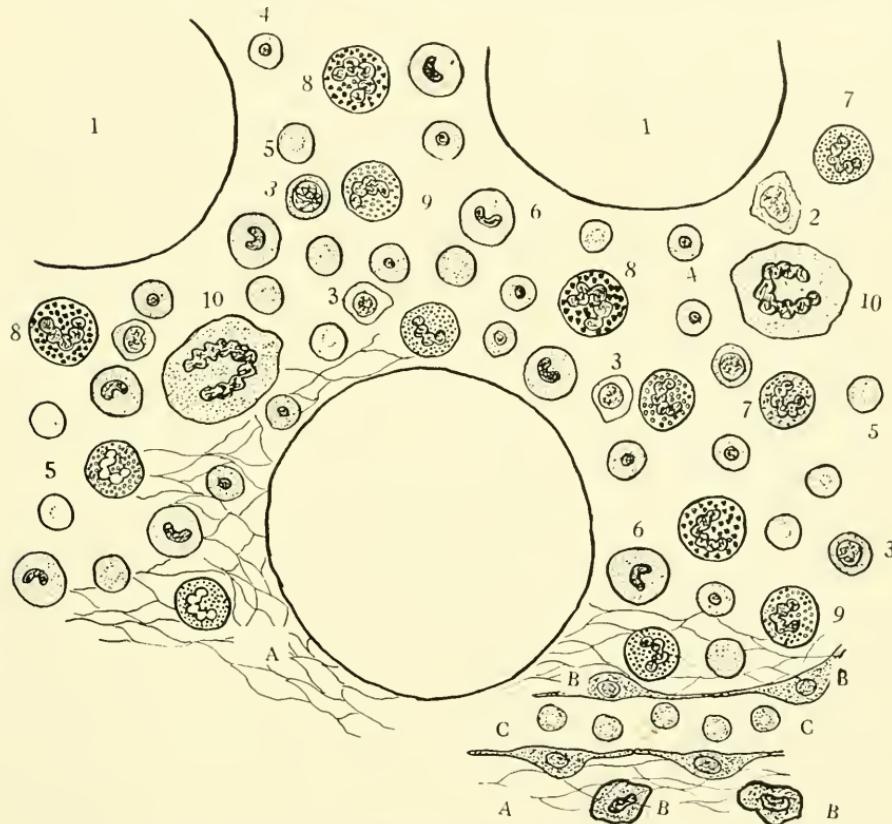
FIG. 42.—Diagrammatic representation of the components of red bone-marrow. *1*, fat cell; *2*, hemocytoblast; *3*, erythroblast; *4*, normoblast; *5*, erythrocyte; *6*, myelocyte; *7*, neutrophil; *8*, eosinophil; *9*, basophil; *10*, megakaryocyte; *A*, reticular tissue; *B*, cell of sinusoid wall; *C*, sinusoid.

eosinophil myelocytes, and basophil myelocytes are formed. Each of these types undergoes its own special development leading to the formation of completely differentiated neutrophils, eosinophils, and basophils which enter the circulating blood by way of the sinusoids.

Megakaryocytes.—These giant cells are usually spherical in form and are found extravascularly in the red marrow of mammals. They also arise from hemocytoblasts by a series of transitional forms. Normally they remain in the marrow, where they are

believed to form pseudopodial-like processes, the ends of which are
pinched off and pass into the blood stream as platelets. Develop-
ing and degenerating megakaryocytes are to be found in red marrow.

The Destruction of Blood Cells.—The different types of blood cells
apparently have differing periods of life. Some are relatively short
lived, as the erythrocytes of mammals which have an estimated life
of about thirty days, but others may live over much longer periods.
It has been observed that many red cells are phagocytized by histio-
cytes, the macrophages, in the red pulp of the spleen; in certain
pathological conditions so many are destroyed that the red pulp
becomes brown in color. Old granulocytes are also apparently
phagocytized by Kupffer cells, the macrophages in the liver. The
lymphocytes are continually degenerating in lymph tissue and
many are lost as a result of their migration through the epithelial
lining of the alimentary tract and various other ducts.

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See Appendix for general text references.

CHAPTER V.

THE MUSCLE TISSUES.

THE most outstanding functional feature of muscle tissue is the capacity to contract and consequently it plays an important part in all movements of an organism. Associated with the functional features are intracellular thread-like structures, the myofibrillæ, which are considered to be the contractile elements. These are embedded in a more fluid cytoplasm, the sarcoplasm. On the basis of structural differences, smooth muscle, cardiac muscle, and skeletal muscle are distinguished. Both cardiac and skeletal muscle fibrillæ have alternating dark and light cross-striations and are often classified as striated muscle in contrast to the smooth muscle in which such striations do not appear.

SMOOTH MUSCLE.

This type shows a very close association with connective tissue and is widely distributed through the vertebrates, occurring in the wall of the alimentary tract, in the arteries and veins, and in numerous other ducts. It is apparently the least differentiated type of muscle, is involuntary, and appears widely in invertebrates in places where, from vertebrate studies, we would expect to find skeletal muscle. The histological unit is easily identified as the smooth muscle cell which is fusiform in shape, though varying greatly in length and breadth.

Embryologically, mesenchyme cells in the region where smooth muscle will develop begin to elongate. A multiplication of such cells, called myoblasts, give rise to a network and finally sheets of smooth muscle cells are formed. The reticular and loose fibroelastic connective-tissue network associated with these differentiating and later fully developed muscle cells is derived from mesenchyme cells similar to those giving rise to the myoblasts. Even in the adult vertebrate it is believed that smooth muscle cells may be derived from undifferentiated mesenchyme cells in connective tissues.

The myoblasts become more and more elongate with development and appear still connected laterally by cytoplasmic processes, as

6

were the mesenchyme cells from which they were derived. Long
threads, the myofibrillæ, appear enmeshed in the sarcoplasm. The
coarse fibrils that appear in the embryonic stage apparently undergo
longitudinal splitting and give rise to more numerous and much finer
fibrils of the fully differentiated cells. Also the lateral connections
are no longer apparent and the cells appear as independent units
structurally, though not functioning as such. The fully developed
cells are fusiform and have an elongated oval nucleus occupying a
central position. (Fig. 43.) The size of the cells varies in different
species and in different regions in the same species.
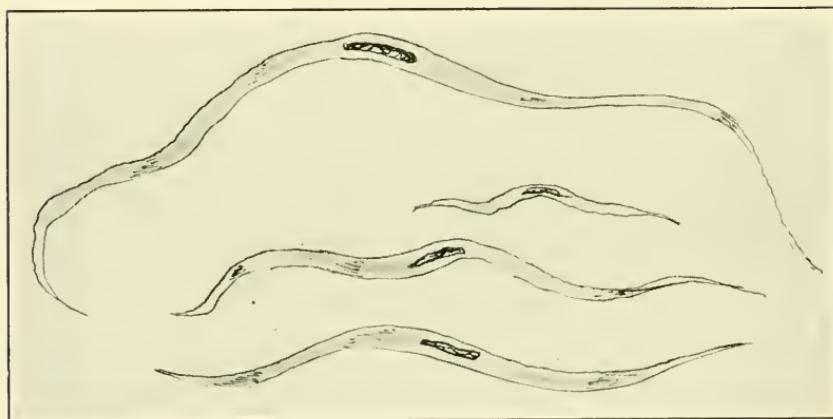
FIG. 43.—Diagram of isolated smooth muscle cells. (Churchill.)

In the mature organization the cells are connected with each other
by a cement substance possibly derived from the former connecting
protoplasmic strands. Although none of this cementing material
is evident in ordinary routine preparations a reticulum of fine fibers
may be demonstrated by silver techniques. Increase in cells may be
effected through development of embryonic cells left among the
mature cells or by division of the mature cells which apparently
retain their ability to divide by mitosis. In newly formed tissue
the fibrils appear to continue from cell to cell, but this cannot be
seen in preparations of older tissues. In general the myofibrillæ
of this type of muscle are difficult to distinguish. The superficial
myofibrillæ appear coarser than those in the interior of the cell.
The non-fibrillar sarcoplasm is best seen as a lighter area at either
end of the nucleus where the myofibrils diverge in passing. By
soaking pieces of tissue composed of smooth muscle in weak acid or

alkaline solutions it is possible to dissolve the substance cementing adjacent cells and shake them apart, so that isolated smooth muscle cells may be observed.

Smooth muscle cells are variously organized. In the connective tissue of the villi or folds of the intestine a few isolated smooth musele cells occur; associated with hairs are small bundles which form the arrector pili muscles; in more complex organizations, the cells are arranged in bundles or sheets. When organized into groups or muscle coats the tapering portions of the cells overlap and a network of elastic and reticular fibers extends between adjacent cells from the surrounding fibroelastic connective tissue. When such sheets or bands of smooth muscle are cut in longitudinal section (Fig. 44), the characteristic spindle shape of the cells is
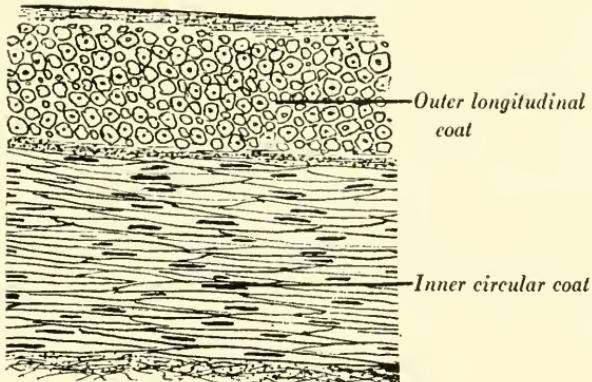

FIG. 44.—Diagram of cross-section and longitudinal section of smooth muscle.

evident, but in cross-sections roughly circular sections of different sizes appear adjacent to each other and nuclei appear only in those sections through the central region of the cells. This is what one would expect from overlapping of the tapering ends.

In the wall of the alimentary tract from the lower esophagus to the anus or cloacal opening there are usually two distinct sheets of smooth muscle; an inner coat of muscle cells encircle the tube, and an outer coat has cells arranged lengthwise. (Fig. 45.) Constrictions of the inner coat decrease the lumen, and constrictions of the longitudinal coat cause a shortening of the tube at the points affected. During life, waves of contraction pass along these coats simultaneously and cause the peristaltic movements essential in the functioning of the digestive system.

Sometimes when pieces of the intestinal wall are fixed, indications of the contraction wave have been preserved. Small band-like swellings running across the muscle sheet appear at regular intervals, these contraction swellings involve different portions of adjacent cells which do not function as independent units. When the intestine or other organ involved is greatly distended, the muscle sheets appear much thinner than in the relaxed state. With relaxation after such expansions the cells slide back into their former position and form a thicker coat. Capillaries extend through the connective tissue network surrounding the muscle cells and follow their
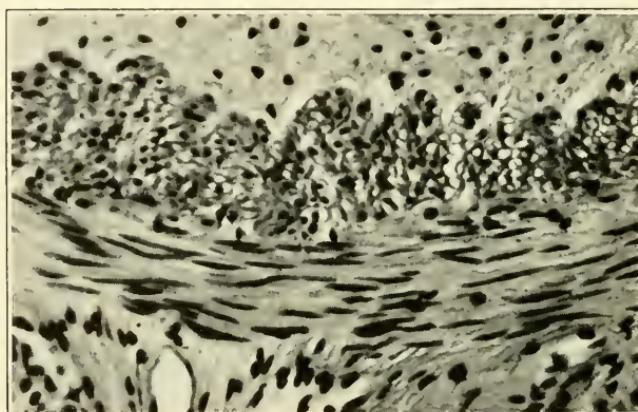

Fig. 45.—Photograph of cross-section and longitudinal section of smooth muscle in wall of frog's stomach.

disposition as do the sympathetic nerves controlling their involuntary action. In the repair of smooth muscle there are evidences of some degree of mitotic activity on the part of fully formed cells, but in cases of extensive injury lesions are closed by scars of connective tissue.

CARDIAC MUSCLE.

Beneath the foregut of the embryo, an endothelial tube surrounded by mesenchyme is the forerunner of the heart and the base of its connecting vessels, to which this type of muscle is limited. The processes of the early mesenchyme cells appear to continue with the adjacent cells to form a syncytium, and from these the cardiac muscle is derived by continued multiplication, gradual elongation, and differentiation of intracellular fibrils. The developing cells, or myoblasts, elongate and granules are said to appear first in the

peripheral cytoplasm. These granules increase in number and appear to become arranged in linear fashion and fuse to form coarse fibers, the cardiac myofibrils. The cells grow in length but preserve their fiber-like form and lateral attachments with adjoining myoblasts. (Fig. 46.) The myofibrillæ increase in number presumably by longitudinal division and become more numerous in the peripheral region, leaving a central portion with a core of undifferentiated cytoplasm and the nucleus. With development the fibrils show striations resulting from alternate differences in composition; these appear as dark and light bands which occur at the same level in all the fibrils of a given fiber, so that the whole fiber has a striated appearance. In the lower vertebrates the myofibrils are less numerous and form a peripheral layer, but in higher forms they are scattered throughout, except in the immediate region of the nucleus.

Fig. 46.—Photograph of an isolated portion of cardiac muscle of the frog, showing a fiber with a central nucleus, striated myofibrils, and branches.

The fibers of higher vertebrates are limited by a thin membrane called the sarcolemma, which is usually considered to be a condensation of sarcoplasm. Among fishes and amphibians, cardiac muscle appears to lack the interstitial tissue found in higher forms, and the bundles of muscle fibers are separated from the blood only by the covering endothelium, a condition resembling the embryonic state of higher forms.

A longitudinal section of cardiac muscle does not have the appearance of separate cells as does smooth muscle. The general picture is that of a network of long fibers in which the nuclei are at regular intervals in the center of the fibers. The clefts between fibers are small in the higher vertebrates but are easily seen in lower forms, such as the fish and frog. A cross-section shows sections of the fibers irregular in outline in places where branching occurs, but in other portions of the fibers the size is more uniform and the outline quite regular. This is in contrast with the marked variability in cross-sections of smooth muscle, where the outlines are regular but the size varies with the region of the cell cut. After treatment in

dissociating fluid, cardiac muscle can be shaken into separate units resembling single cells. They are roughly rectangular in shape, with parallel sides and very uneven ends. One or both ends may have short stubby branches that connected it with other adjacent fibers. Each of these units is cross-striated and shows longitudinal fibrillæ. It is not certain that the apparent ends are actually boundaries of cells; they may be artefacts brought about by the treatment.

The isolated units of cardiac muscle correspond to cells having a nucleus and a surrounding portion of cytoplasm. However, the most reasonable conclusion drawn from the lack of definite cell boundaries, and the fact that the myofibrils extend continuously through several such units, is that the cardiac muscle is a syncytium. The myofibrils appear to be similar to those of skeletal muscle,
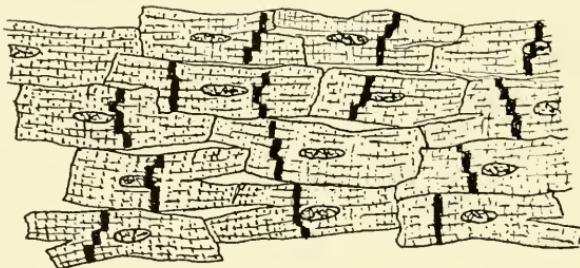

Fig. 47.—Diagram of cardiac muscle cells, showing intercalated discs.

where they are more easily studied. At times even in fresh heart tissue, and particularly after certain techniques, peculiar bands, the intercalated discs, may be observed extending partially or interruptedly across a fiber. (Fig. 47.) These discs were formerly thought to represent end-boundaries of the cells, but this seems unlikely since some of them pass across a fiber at the level of the nucleus and others delimit portions of a fiber without a nucleus. They usually present a staircase appearance and the separate portions do not overlap. Some investigators interpret them as places where the fibers were in a state of contraction. They are more commonly found in older cardiac tissue and from this the inference has been drawn that possibly they represent lines where normal functioning is breaking down. The exact nature of these structures remains to be demonstrated.

The blood supply for cardiac muscle is supplied directly from the clefts between the fibers in the case of the lower vertebrates,

as may be observed in the fish or amphibian heart. In mammals, however, there is a rich capillary network carried between the fibers by the interpenetrating connective tissue. The syncytial organization of the heart is probably directly concerned with the rhythmical contractions so characteristic of it, but it is also essential that a free circulation takes place regularly, for interferences in the blood flow affects the normal rhythmical activity. Cardiac muscle contractions are shorter in duration than the resting phase and under normal conditions fatigue rarely occurs. Regeneration does not seem to be possible in cardiac muscle and enlargements of the heart occurring in some adult animals result from increase in the size of the muscle fibers, not their number, or possibly from increase in the connective tissue if present.

Neurogenic and Myogenic Theories of the Heart-beat.—Although this appears to be primarily a functional problem, histological discoveries have had much to do with our knowledge of the subject. The heart of an elasmobranch is two-chambered; there is one auricle and one ventricle. The sinus venosus draining blood from the body carries this blood into the auricle; from the latter, the blood passes into the ventricle whose contraction drives the blood into circulation through the body. In action, the sinus contracts first, then the auricle, then the ventricle, then the bulbus arteriosus. In this order, one after the other, repetition occurs rhythmically. It appears that whatever the nature of the stimulus is, it begins in the sinus wall. The amphibian heart has two auricles and one ventricle; reptiles have two auricles and the beginning of two ventricles; birds and mammals have two auricles and two ventricles. In each case the old elasmobranch heart organization is represented roughly by tissue at the junction of the vena cava with the right auricle, and careful observation shows that the rhythmic contraction of these higher hearts begins at this tissue and is followed by contraction of the auricles and ventricles.

The heart is provided with branches of sympathetic nerves which have a sensory function, and with branches of the tenth cranial and the vagus over which the impulses regulate the speed of the rhythmic beat. There is no evidence, however, that either set of nerves is concerned with the origin and continuance of the rhythm. Rhythmical contractions of so-called hearts of invertebrates are effected by nerve impulses, and the occurrence of nerves in vertebrate hearts suggested that the heart-beat was due directly to nerve stimuli. However, this explanation does not apply to vertebrate hearts.

The embryo heart beats rhythmically before nerves have developed in it, and when the heart of a cold-blooded vertebrate is removed from its body, rhythmic beating may be continued for many days if proper conditions are maintained. Also, small pieces of heart of the chick or rabbit live, grow, and contract in tissue culture after the nerves have degenerated. Evidence is still needed to prove the neurogenic theory of the heart-beat.

The myogenic theory, on the other hand, has much in its favor. According to this idea, the stimulus arises in cardiac tissue and is transmitted by this tissue to various contracting portions. The question then arises as to whether impulses pass over ordinary muscle fibers or whether there are special fibers for this function. Such fibers have been found in mammals, where a small mass of especially modified fibers occur at the junction of the superior vena cava and the right auricle. These fibers are poor in fibrils but rich in sarcoplasm; they are small, poorly striated, and form a network. This mass is called the sino-auricular node and is the place where the automatic rhythmic contractions begin. The impulses continue over the auricle *via* a network of the same type of fibers, the so-called Purkinje fibers, which become continuous with typical cardiac fibers. The auricles are separated from the ventricles by rings of connective tissue around the openings between them and Purkinje fibers converge there to form the auricular-ventricular node, a second mass of modified cardiac fibers. This node is located near the ventricles and Purkinje fibers extend on into the ventricles. Impulses beginning at the junction of the vena cava with the auricles cause the latter to contract, and then the impulses continue into the ventricles, causing a progressive contraction in them also. The rest period between each cycle of contraction is longer than the contraction period. The heart is refractory during this rest period and it does not respond to stimuli. Its sensitivity to stimuli increases toward the end of the rest period. If the impulses to the ventricles are interfered with, the ventricles do not respond, or may set up an independent rhythm of their own, in which case the heart as a whole fails to function properly.

SKELETAL MUSCLE.

As the name implies, this type is associated with skeletal parts. The unit of structure is the fiber, as it was in cardiac muscle, but here the fibers are unbranching, elongated, multinucleated cylinders of varying length. Such fibers likewise are formed from myoblasts

which are derived from mesodermal cells. Though not branching
and forming networks, as in the case of cardiac muscle, they are like
it in being syncytial. Distinct cellular limitations are not evident
and many nuclei are distributed along the length of each fiber. These
fibers do not break into semblances of cells upon treatment with dis-
sociating fluids. The development of the elongate multinucleated
fibers is held by some to occur by repeated divisions of the nuclei
of myoblasts without accompanying division of the cytoplasm,
which increases in quantity and elongates. Others believe they
arise through fusion of the ends of adjacent myoblasts. Fibrils
appear first about the periphery and increase in number and dis-
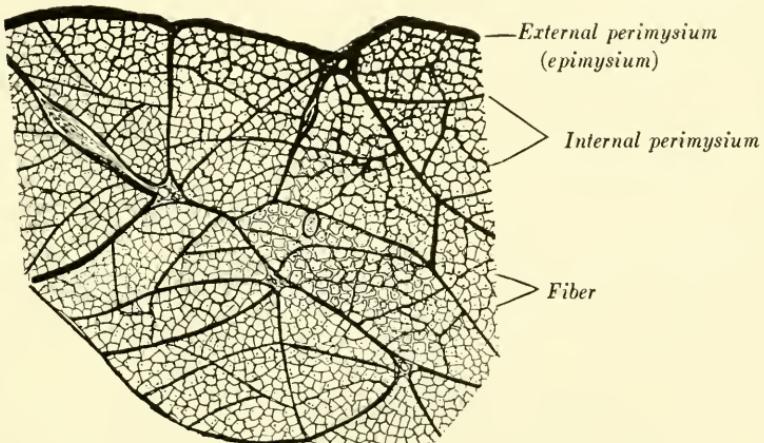
FIG. 48.—Diagram of a cross-section of a skeletal muscle.

tribution during development. The nuclei of the myoblasts at the
first appearance of fibrils are central, but later in development they
appear more peripherally located.

The fibers are organized into skeletal muscles which are enclosed
in a relatively thick fibroelastic connective-tissue sheath. (Fig. 48.)
This is the external perimysium (epimysium) which continues inter-
nally as the internal perimysium to divide the muscle into bundles of
fibers, or fasciculi. (Fig. 49.) The individual fibers are surrounded
by a thin sheath of connective tissue, called the endomysium,
which contains fibrous and cellular elements of loose fibroelastic and
reticular connective tissues. (Fig. 50.) Fibrocytes and histiocytes
and undifferentiated mesenchymal cells in the connective-tissue
sheaths play a part in the repair of lesions of skeletal muscle which
does not exhibit marked powers of regeneration. The capillaries

and nerve fibers as well as the larger blood vessels and nerve trunks
are supported in the connective-tissue networks surrounding the
individual fibers and the fiber bundles.
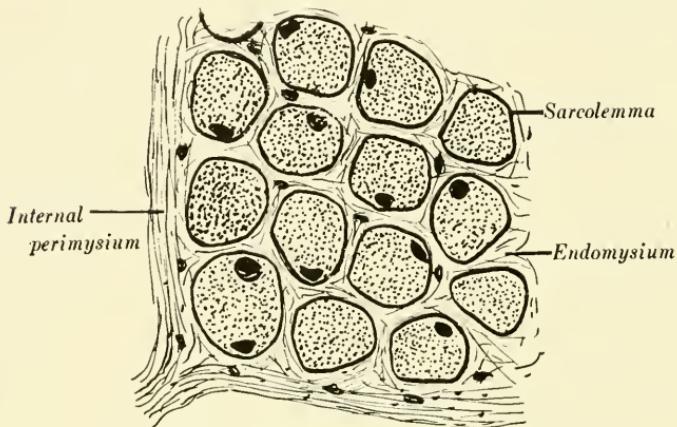
FIG. 49.—Diagram of a bundle of skeletal muscle fibers.

Individual fibers may be observed in fresh skeletal muscle by
teasing with needles, but it is difficult to find uninjured ends of
such fibers. In fixed and stained preparations, fibers are seen to
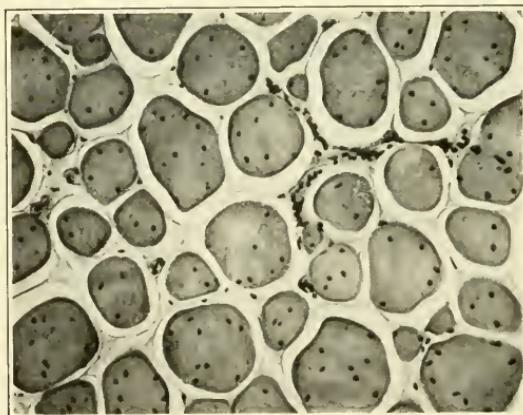
FIG. 50.—Photograph of a cross-section of skeletal muscle of the turtle, showing fibers
surrounded by endomysium. Capillaries may be seen between some fibers.

end in various ways. Some are rounded and blunt, others taper off
within their connective-tissue sheath, which becomes continuous
with other sheaths to which the fiber attaches within the muscle or

with tendinous tissue with which it is thus attached to bones, carti-
lage, or other structures. The fibers are clearly limited within their
endomysial sheath by a continuous, thin, transparent membrane,
known as the sarcolemma. In injured regions of teased fibers the
fibrillar contents are often broken and separated, making it possible
to observe this membrane more easily. Its origin is disputed; some
believe it to be formed by the connective sheath, and others
attribute its presence to the activity of the protoplasm of the
muscle fiber itself. The latter seems more likely, for it has been
shown to have none of the reactions of collagen or reticulum of
connective tissue.

Within the sarcolemma the fibers are composed of a fluid proto-
plasmic substance, the sarcoplasm, and numerous highly developed
myofibrils which run parallel to each other lengthwise of the fiber.
The fibrils appear to originate, as in the case of those in cardiac
muscle, from linear fusion of fine granules forming in the embryonic
myoblasts. Contraction first appears when the myofibrils have
formed. They increase in number with development of the early
fiber and are arranged into groups separated by intervening sarco-
plasm. Such groups of fibrils are called sarcostyles and are apparent
in cross-sections as Cohnheim's areas. The separation of such
groups from each other depends upon the amount of intervening
sarcoplasm; in some muscles they are not easily discovered. Fixa-
tion may also play some part in producing these decidedly localized
groups of fibrils.

Although each fibril is a continuous thread of protoplasm, it
appears to be composed of plates, or discs, of two alternating kinds
of material. These give rise to the dark and light bands forming the
cross striations characteristic of this and cardiac muscle. The
discs show better when the muscle tissue is soaked in dilute aqueous
solutions of acids and alkalies. A number of bands have been
identified, but only four are easily demonstrated and ordinarily
only two of these are outstanding. The relatively broad, dark,
refractive band stains with hematoxylin and is called the "Q" or
anisotropic band. (Fig. 50a.) Alternating with these are the less re-
fractive, pale discs that ordinarily remain unstained; these are the
"J" or isotropic bands. Each of these bands is apparently divided by
another narrower band of opposite character. The "Q" band is
thus seen to have an indistinct light band, the "M" band, running
through its center; and the "J" band has an indistinct thin dark
band, the "Z" band, or intermediate disc, running through its

center. These latter bands represent differences in the densities of the "*Q*" and "*J*" bands. The region between two "*Z*" bands is considered to be a functional and structural unit, the sarcomere, of the fibril. Various theories of muscle contraction have arisen, based on the different peculiarities associated with the parts of these sarcomeres, but little is really known of the mechanism of contraction. The dark "*Q*" discs are doubly refractive, poor in extensibility, poor in water content, do not shrink, and stain darkly in hematoxylin. The lighter "*J*" discs are singly refractive, pale, rich in water content, extensible, shrink in reagents, and do not stain well, if at all.
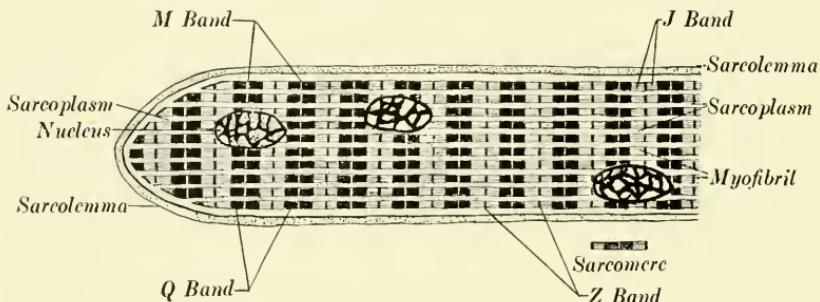

Fɪɢ. 50*a*.—Diagram of a skeletal muscle fiber in longitudinal section.

The nuclei of skeletal muscle fibers are oval in shape, but their size and location varies, depending upon the animal. In lower vertebrates the nuclei occur scattered throughout the fiber among the fibrils, but in some cases show a tendency to be located more abundantly near the periphery. In mammals the nuclei are most commonly located in the sarcoplasm, directly below the sarcolemma, but in some muscles of the red type they may be scattered more generally through the fiber. The nuclei vary from an oval to a fusiform shape, but are disposed lengthwise in the fiber in either case.

The sarcoplasm filling in between the fibrils may vary greatly in amount and the density and size of the fibers also show variations with species. There is usually an accumulation of sarcoplasm about the nuclei, especially at either end. Variations in the size of muscles in mature animals is dependent mainly upon increase in sarcoplasm.

The nerve supply from the cerebrospinal nerves places this type of muscle under voluntary control in contrast to the two other types of muscle. Capillaries form a meshwork with the longer capillary portion running lengthwise of the fibers, and short and

thicker cross-pieces, or ampullæ, extend across the fibers and may dilate with blood when the muscle is contracted.

If a muscle is seriously injured its destroyed portion is replaced by scar tissue, but if the sarcolemma of a fiber is not injured the injured myofibrils are digested and the fiber remaining acts like a myoblast and forms new nuclei and fibrils, possibly also new units by mitosis.

The manner of attachment of skeletal muscles to tendons is thought by some to involve a continuation of the myofibrils with the collagenous fibrils of the tendon, by others to involve continuations of the connective tissue (endomysium) with the tendon. The latter appears to be the more likely association.

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See Appendix for general text references.

CHAPTER VI.

THE NERVE TISSUE.

ALL cells are to a certain extent irritable and conductive; that is, they receive stimuli from external sources and transform them into impulses which are conducted to a portion or the whole of the cell to stimulate some reaction by that portion or by the entire cell. In unicellular animals and the simplest metazoans, no special organization appears to be developed to carry on these fundamental functions of protoplasm. Among the metazoans generally, however, an association of special cells has evolved to form the nerve tissue, which functions primarily as a receiver of stimuli and conductor of impulses. The vital unit of this tissue is the nerve cell, or neuron, which takes on varied forms but invariably has one or more cytoplasmic processes making contact with closely adjacent or more remote cells or tissues of the body. Typically, each cell has a large nucleus surrounded by a cytoplasmic mass from which slender processes grow out for varying distances to form nerve fibers. Each neuron is a separate unit, but the processes of one cell come into contact with others at synaptic junctions, or synapses, so that impulses pass from one nerve cell to another, and by chains of such cells impulses may be conducted over considerable distances to finally effect responses in other cells or tissues. Little is known concerning the exact nature of stimuli, or how they are transformed into impulses, or how the latter are transmitted along nerve cells, but the essential part played by these cells in coördinating the other tissues of the organism has been proven repeatedly by careful experimentation.

The nerve cells and their processes are organized into organ centers such as the brain, spinal cord, and ganglia, but the processes alone form the nerves which are organized into an interconnecting system associating the various tissues and organs with the nerve center and making possible integrated action.

HISTOGENESIS OF NERVE TISSUE.

The foundation of all the nerve tissue appears in the developing embryo as a thickened region of ectoderm, the neural plate, along the mid-dorsal line. Following rapid and unequal growth of the

cells of this plate, a neural groove is formed and deepens until the thickened folds fuse dorsally to form a neural tube which lies below and separate from the ectoderm. The cells of this neural tube give rise to the major part of the nerve tissue. The anterior portion of the tube forms the brain and the posterior portion the spinal cord. Between the neural tube and the ectoderm a longitudinal band of cells appears on each side and forms the neural crest. From these cells are derived the spinal and cranial ganglia and indirectly the sympathetic ganglia. The cells of the neural tube undergo a series of divisions and finally differentiate into the nerve cells or neurons and the neuroglia cells which form the supporting tissue of the nervous system.

THE NEURON.

A study of nerve tissue involves an understanding of the neuron, or nerve cell. A description of it includes a consideration of the cyton, which consists of the nucleus and surrounding portion of cytoplasm, and the cytoplasmic processes which extend out from the cyton. (Fig. 51.)

The Cyton.—The size of this portion of the neuron varies from a few to several hundred microns in diameter. The limiting surface is a denser cytoplasm but not a distinct cell membrane, as found in other types of cells.

Fig. 51.—Diagram of a neuron.

There is considerable variation in shape, some cell bodies are spherical, others oval, pyriform, fusiform, or even stellate. Such variations are largely due to the number and location of the processes which extend out from the cytons.

Nucleus. — The large spherical nucleus is bounded by a distinct nuclear membrane. Within it is a large nucleolus which stains intensely with basic dyes. The chromatic material within the nucleus is generally scant, as compared with that of other cells. In large cytons it appears as a network concentrated about the nucleolus. Fine protoplasmic granules staining with both basic and acid dyes occur abundantly. In some neurons several nucleoli may be observed. Mature neurons differ from other cells in that they do not undergo mitosis.

Nissl Bodies. — These irregular masses of basophilic material occurring in the cytoplasm have been called tigroid bodies, chromophil substance, and other names. (Fig. 52.) Special techniques
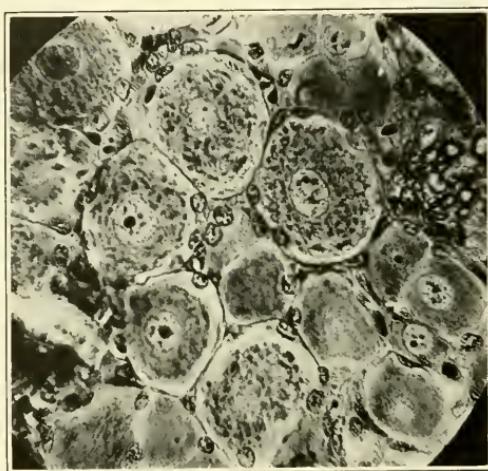

Fig. 52.—Spinal ganglion cells of the cat, showing numerous Nissl bodies. Each cell is surrounded by a number of smaller satellite cells.

are often used to make them stand out clearly, but almost every stained preparation shows them scattered through the cytoplasm. It is believed that these bodies contain iron and store oxygen, and that their presence is essential to the functioning of the nerve cell. Their appearance as definite bodies may be due to fixation, since different fixatives result in differences in their form. In the living cells the substance of which they are composed may be in a diffuse state. Modifications in their appearance occur with changes in the conditions of the nerve cells. In pathological conditions they often disappear entirely.

Neurofibrils. — In living cells it is difficult to demonstrate anything but a more or less homogeneous cytoplasm, but in fixed and stained preparations, especially silver impregnated tissues, very fine long thread-like structures appear to extend throughout the cell body and its processes. At first they were thought to be the primary means by which impulses were transported, but there seems to be little proof as to their function, though they appear as characteristic features in nerve cells.

Golgi Apparatus. — By special techniques, using osmic acid and silver, a blackened network appears in the cytoplasm usually concentrated about the nucleus. This apparatus of Golgi disappears in cells subjected to injury and cannot be demonstrated in living unstained cells.

Chondriosomes (Mitochondria). — Tiny rods and granules appear scattered through the cytoplasm and may be demonstrated in living cells with Janus green B. Certain methods also preserve them in fixed and stained preparations. Little is known of their functional significance.

Cytoplasmic Processes. — It is customary to recognize two types of processes extending from the cyton, namely, axon and dendrites. There is no Nissl substance in the axon. It is a slender fiber-like extension of uniform diameter throughout and has a smooth clean surface. Usually the axon arises from a particular place in the cyton marked by a conical extension called the axon hill. Slender collateral branches may arise from the axon along its course and are at right angles to its surface. In many neurons the axon ends distally in a brush of finer branching processes known as the terminal arborization or telodendria. The dendrites are thick, irregularly branching, cytoplasmic processes extending out from the cyton and contain the Nissl substance and other cytoplasmic elements generally present in the cyton.

THE REFLEX ARC.

The simplest physiological organization of neurons is called a reflex arc. It involves at least two neurons, as in the following example. A stimulus on the skin is translated into a nerve impulse in the peripheral terminus of a dendritic process of neuron A. (Fig. 53.) The nerve impulse travels centrally to the cyton of this neuron, which is located in a spinal ganglion. From this cyton the nerve impulse passes over its axon into the gray matter of the cord to terminate in the ventral horn. Here it connects with the den-

7

drites of neuron B, and passes into the cyton of this second nerve
cell. From cyton B the impulse travels into the axon which extends
outward in the spinal nerve to muscle tissue and stimulates the
muscle to contract. Most reflex reactions involve more than two
neurons. This is especially true of higher mammals, where volition
exercises control over simpler reflex mechanisms.
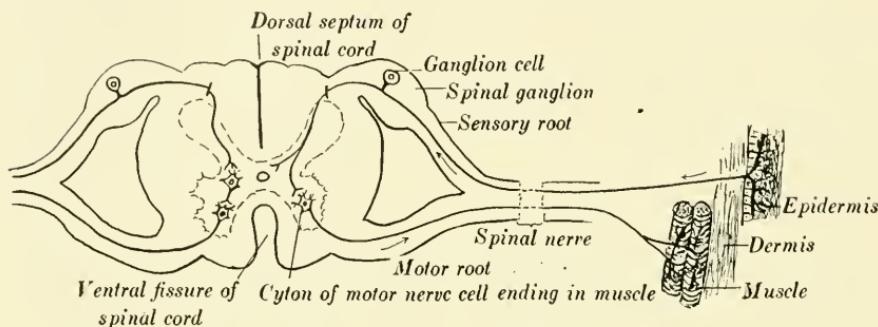
Fig. 53.—Diagram illustrating a reflex arc.

THE SYNAPSE.

Neurons are associated with each other through the synapse, at
which point the terminals of the axon of one neuron come into
contact by contiguity with the dendrites or cell body of another
neuron. Such association may be effected by a basketwork of
neurofibrillar processes from the terminal end of one neuron fitting
against the dendrites or cyton of the neuron receiving the impulse.
The synaptic type of association is accepted as the usual one, but
there is evidence showing that nerve fibers may be continuous at
these points, especially among the lower vertebrates.

TYPES OF NEURONS.

Neurons located in different regions of the nervous system have
definite functional demands made upon them, and associated with
their functioning is a certain arrangement of their processes so that
several types of cells may be classified as follows: unipolar cells,
neurons with a single process which arises from one side; bipolar
cells, in which a single axon and a single dendrite process project
from opposite ends of the neurons; multipolar cells, in which numer-
ous processes project from different regions of the cytons; and
ganglion cells, in which two different processes, axon and dendrite
arise from one side, a pseudo-unipolar condition.

Unipolar Cells.—During the early differentiation of the neuroblasts, a unipolar condition develops when a single process grows out from the cell body. This condition does not usually remain throughout development, for one or more additional processes are developed from different portions of the cyton, and change the cell into a bipolar or multipolar type when differentiation is complete. In some lower vertebrates the unipolar condition is believed to remain in completely differentiated neurons of the brain, spinal cord, and ganglia. In such cases there is some uncertainty as to whether the single group of similar processes arising from one region of the cell represents an axon or dendrite, though in some in-

Fig. 54.—Photograph of a pyramidal cell from the cerebral cortex of a cat with two protoplasmic astrocytes surrounding the largest dendritic process. The axon leaves the base of the pyriform cell body.

Fig. 55.—A photograph of a Purkinje cell from the cerebellum of the cat, showing much branched dendritic processes and a single fine axon process. Golgi technique.

stances both axons and dendrites may be differentiated from part of the process.

Bipolar Cells.—In these neurons a single axon and dendrite are developed and project from opposite ends of the cyton. This condition is found in the embryological development of the spinal and cranial ganglion neurons before they are completely differentiated, and is true of completely differentiated neurons found in the retina and parts of the ear.

Multipolar Cells.—This type of cell is by far the most numerous and the most easily demonstrated. Although beginning its development with a single cytoplasmic outgrowth, it eventually develops one axon process and several dendritic processes. The shape of these cells is, therefore, dependent upon the number and arrangement of the dendrite processes. Examples are found in the pyramidal cells of the cerebrum; the Purkinje cells of the cerebellum; and the motor cells in the ventral horn of the spinal cord.
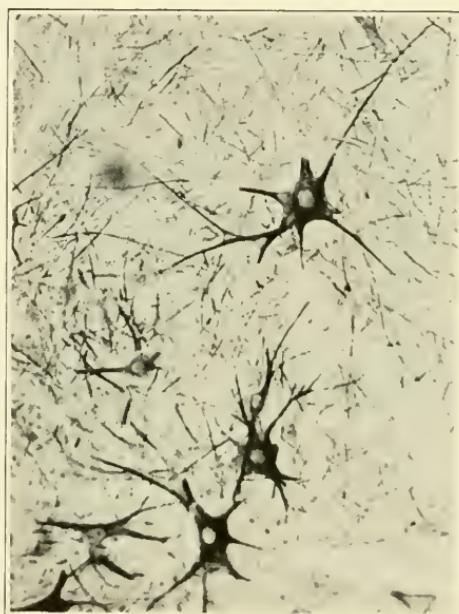
Fig. 56.—A photograph of multipolar cells from the ventral horn of the cat's spinal cord. The nucleus occupies the light central area of each. Cajal method.

Pyramidal cells are characteristic of the cerebral cortex. (Fig. 54.) The cell body has a pyramidal shape with a long thick branching dendrite extending from the narrow end and a number of shorter dendrites arising from the sides and base. A single slender axon arises from the base and extends down into the white matter of the brain. Another variety of this cell has a short axon which branches near its origin and extends only a short distance from the cell body, a condition which has led to considering them association cells.

Purkinje cells are characteristic of the cerebellar cortex of mammals. (Fig. 55.) The cytons of this type are pyriform, but have

only one or two main dendrites which subdivide to form a thick bush-like thicket of processes. An axon arising from the base of the cell body extends into the white matter.

Motor neurons of the spinal cord have irregularly stellate shapes due to the origin of dendrites from many points. (Fig. 56.) The axon is a single, slender, and smooth process of the cell body and often extending long distances.

Craniospinal ganglion cells have a superficial appearance of unipolar conditions. The cell body is globular or pyriform, and has a single process which has the characteristics of an axon. Embryologically the cells produce two processes, a bipolar condition, but later the processes fuse in a common outlet. This single process usually branches to form two axon-like processes, one extending to the periphery, regarded as a dendrite, and the other passing into the dorsal horn of gray matter of the cord and regarded as an axon. Surrounding each of the ganglion cells is a sheath of smaller satellite cells, or cells of Schwann, since they are believed to continue out over the process to become continuous with the sheath of Schwann covering the fiber. (Fig. 52.)

THE NERVE FIBER.

As the axons of neurons pass into the white matter from the gray matter of the cerebrum, cerebellum, and spinal cord, they are clothed
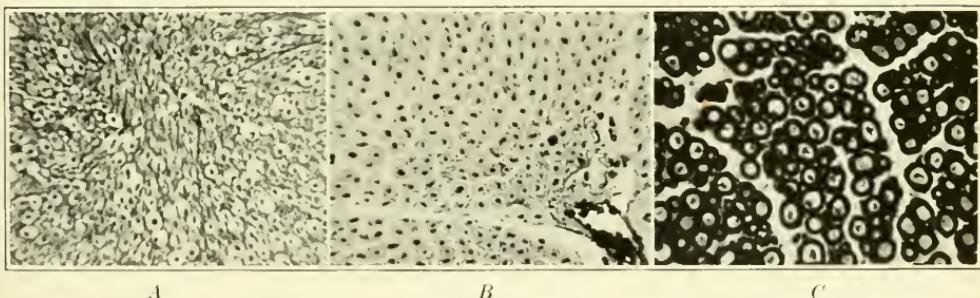
Fig. 57.—Cross-sections of myelinated peripheral nerves, showing appearance following different treatments. *A*, fixed in Bouin; stained in eosin and hematoxylin. The myelin is dissolved away. *B*, prepared with silver impregnation to show the axis cylinder. *C*, fixed with osmium tetroxide and preserving the myelin.

with a lipoid substance, called myelin. (Fig. 57.) As myelinated axons pass from the brain and cord to become the components of cranial and spinal nerves, a sheath of Schwann, or neurolemma sheath, is added. This consists of a successive series of flat, nucleated cells

wrapped around the myelin coating. These cells vary in length,
being longer and larger in the case of axons of large neurons.
(Fig. 58.) Where the ends of these cells meet along the fibers, the
edges appear to be in contact with the axon, so that at these depres-
sions, or nodes of Ranvier, the myelin is interrupted. The portion
between two adjacent nodes is called the internode and represents
the length of the neurolemma cells. Between the nodes of Ranvier
the myelin sometimes exhibits funnel-like interruptions, called
incisures of Lantermann, which some observers regard as artefacts.
In the middle of each internode, at some place on the internal
periphery of the neurolemma, is a nucleus surrounded by a small
amount of granular protoplasm. Some histologists regard the
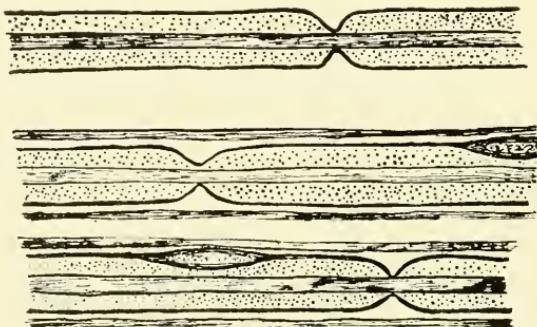
Fig. 58.—Diagram of three nerve fibers. Endoneurium is shown around the two
lower fibers. A central axon is surrounded by myelin (dotted) which in turn is
surrounded by neurolemma. The nucleus of the neurolemma is shown in lowest
fiber. Each fiber has a node of Ranvier.

neurolemma and myelin between each pair of internodes as a single
cell. The peripheral terminations of motor axons branch into arbori-
zations about which myelin is lacking. Outside of the neurolemma
of most peripheral nerves is a closely fitting sheath of delicate
argyrophil fibers called the sheath of Henle.

In the central nervous system some fibers appear to be embedded
in neuroglia but lack myelin. Furthermore, many axons of the
nerves from sympathetic ganglia have no myelin and are surrounded
by a sheath of neurolemma in which there are no nodes of Ranvier.
These are known as fibers of Remak.

HISTOLOGY OF A PERIPHERAL NERVE.

Peripheral nerves originating from the brain and spinal cord
emerge through foramina of the skull or vertebræ and pass to out-
lying parts. Such a nerve consists of a great many hundreds of

fibers, each composed of an axon, myelin, neurolemma, and sheath of Henle. The fibers are arranged in bundles, or fasciculi, of varying size, bound together by loose fibroelastic connective tissue, and the entire nerve is supported by similar tissue. (Fig. 59.) The connective tissue immediately around all the fasciculi is called epineurium, or external perineurium, and as the internal perineurium it continues in between the bundles and merges with a more dense connective tissue around each bundle. There is also a loose delicate
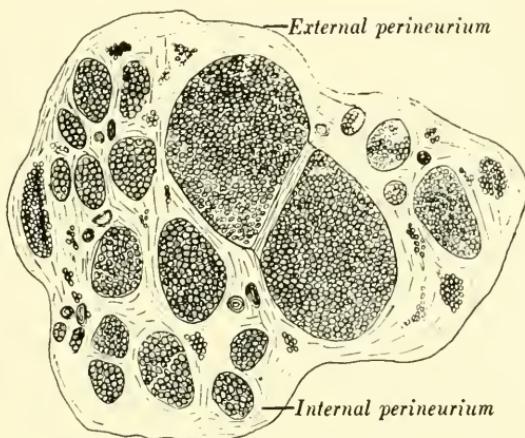
Fig. 59.—Drawing of cross-section of a peripheral nerve. Two large and a number of smaller fasciculi of fibers are shown. External and internal perineurium are shown around and between the bundles.

extension of connective tissue between adjacent single fibers to form the endoneurium. Delicate fibers from the endoneurium continue to the sheath of Henle.

As the nerve extends out from its central origin, it branches, and each branching represents a sorting out of bundles and a gradual diminution in number of fibers in a bundle. The connective tissue of a nerve has a special nerve supply, called the nervi nervorum, and a vascular supply, the vasi nervorum.

RECEPTORS AND EFFECTORS.

The reception of external stimuli by nerve cell processes and the transforming of these into nerve impulses necessitate some organization of the other tissues located at each of these end-points. As an example, there are receptors in the eye which receive stimuli from light and others in the skin which receive stimuli from pressure. The

stimuli from such sources arouse nerve impulses which are trans-
mitted by the neurons to others ending in effectors. Such effectors
are organizations of the nerve endings with other tissues which
they stimulate into action The eye and skin are exteroceptors
in that they receive stimuli from outside the body. There is another
group of internal receptors, the enteroceptors, or visceral receptors
which receive stimuli from internal pressure. Still other receptors,
the proprioceptors, supplement the internal and external receptors
and lead to a regulation of the reactions set into play by the impulses
conveyed to the effectors. A few of the many devices of receiving
and affecting stimulation will serve as examples of the interrelation
of the neurons with other tissues. Those nerve endings receiving
stimulation are called sensory; those effecting stimulation of other
tissues or cells are the motor endings.

Sensory Endings.—The free ending of fine nerve branches demon-
strated between and close to epithelial cells are associated with
both sensory and motor impulses. Morphologically sensory and
motor free ends of nerves are often similar.

In glands, the terminal ends of sympathetic fibers form a network
just outside the basement membrane; some branches pass through
this, forming another net around the bases of the gland cells, and
some small branches extend between the gland cells. Some of these
act as receptors and others as effectors regulating secretion. An
arrangement of free endings similar to this is present in stratified
squamous epithelium, as, for example, the epidermis, where it is
sensory. Organs of special sense have epithelial cells, derived from
ectoderm which are especially sensitive to particular types of stimu-
lus. In the upper back region of the nasal passage, among the
protective cells of the membrane, are special olfactory cells which
connect basally with nerve fibers of afferent neurons, forming part
of nerve pathways to the olfactory center of the brain. There are
special cells in the cochlea of the ear which connect with processes
of neurons belonging to the auditory branch of the eighth nerve,
and so form part of a pathway to the auditory center in the brain.
These pick up vibrations which become translated as sounds. The
rods and cones of the retina are stimulated by light waves and
connect with a bipolar cell whose outer ends connect with other
neurons which extend in toward the brain.

Skeletal muscles have sensory end-organs, called muscle spindles.
(Fig. 60.) They are formed about a group of small, weak, pale
muscle fibers, separated by connective tissue from the surrounding

fibers. The distal end of an afferent nerve breaks up into fine
thread-like branches which are coiled about these special fibers.
When the muscle as a whole has contracted, the sensory nerve of
the muscle spindle is stimulated, and this is relayed to a nerve
center. In this way we get information of the position of the limbs,
for example, the degree of flexion.

Distributed widely through fibroelastic connective tissue, are
special sense organs called Pacinian corpuscles. These are small
ovoid structures consisting of concentric overlapping layers of
connective tissue covering an inner core of semifluid plasm in which
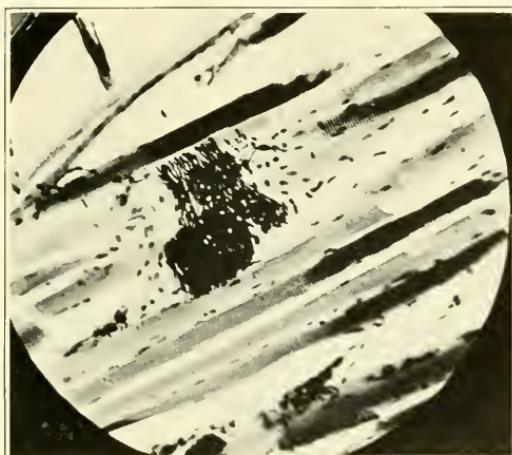
Fig. 60.—Photograph of neuromuscular spindle in skeletal muscle of the cat.
The terminal arborization of a nerve fiber is wrapped about skeletal muscle fibers
and acts as a sensoreceptor. Silver impregnation.

is embedded the flat end-process of a nerve. These are a few of the
numerous types of sensory end-organs.

Motor Endings.—A motor nerve as it ends in a skeletal muscle
passes in through the external and internal perimysia to the muscle
fibers. The end of each nerve fiber breaks up into telodendria and
myelin disappears. The neurolemma at the end of the fiber appears
to merge with the sarcolemma of the muscle fiber to which that
particular telodendrion is connected. The group of neurofibrils
passes into a shallow pool of sarcoplasm just under the sarcolemma,
where the nerve process forms short irregular branches, each ending
in a small knob. The whole apparatus is known as a motor end-
plate. The nerve impulse reaching this organ causes the muscle to
contract. Each muscle fiber has at least one motor end-plate.

NEUROGLIA.

Although the peripheral nerves are supported by connective-tissue frameworks, the nerve tissue of the brain and spinal cord is supported by the special tissue called neuroglia. Additional, less conspicuous mesodermal elements, called microglia, enter into the nerve tissue of these regions with the blood vessels. Arising from the primitive cells of the neural tube, several types of neuroglia cells are differentiated.

Ependyma Cells.—The columnar epithelial-like cells lining the embryonic neural canal develop long processes extending across the wall of the developing tube. In the course of development these processes are lost, so that in the mature animal there remain columnar cells with tapering ends projecting into the tissue of the cord and brain. Cilia are usually present on the free surface of these cells. In certain regions of the brain there are vascular invaginations, called choroid plexes, where the ependymal cells lose their cilia, become cuboidal, and act as a secretory epithelium.

Fig. 61.—Types of neuroglia cells. *A*, protoplasmic astrocyte; *B*, fibrous astrocyte.

Astrocytes.—The astrocytes are stellate cells with processes attached to blood vessels, and are the largest type of neuroglia element. The nucleus is large and oval, with scant scattered chromatin and no nucleolus. Two types are usually recognized, fibrous astrocytes and protoplasmic astrocytes.

Fibrous astrocytes, also called spider cells, are characterized by long, usually unbranched processes containing fibrous elements. They occur abundantly among the myelinated fibers of the white matter of spinal cord and brain. (Fig. 61.)

The protoplasmic astrocytes, which occur in gray matter, have a stellate cell body with granular cytoplasm and many short, stubby, branching processes. The abundance of the processes, as shown in Golgi preparations, has led to calling them mossy cells.

Microgliocytes.—These elements appear as small cells in their resting state and have fine-branched processes, but are capable of becoming ameboid and phagocytic. The nuclei of these cells are the smallest and stain more deeply than those of other cells found in the spinal cord and brain.

The function of the astrocytes is associated with support and healing in the case of injury and possibly assist with the formation of myelin. The microgliocytes may be considered as analogous with phagocytic cells in other regions of the body, and form part of the reticulo-endothelial system of the nervous system.

GANGLIA.

A ganglion may be defined as a small aggregation of neurons (cytons and processes) outside the central nervous system. (Fig. 62.) Each dorsal root of a spinal nerve possesses a spinal ganglion. The dorsal root of such a nerve is sheathed in external perineurium.
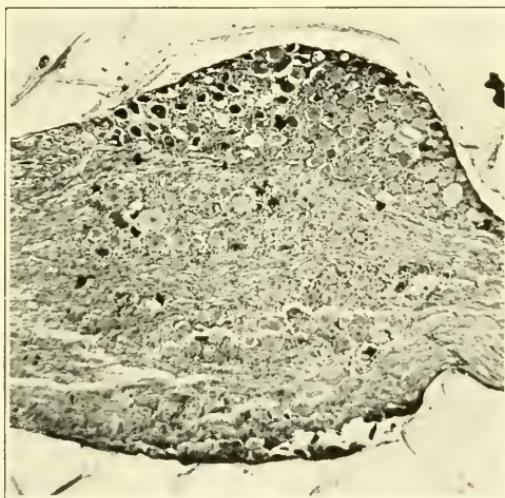
FIG. 62.—Photograph of spinal ganglion of the cat. Longitudinal section showing groups of ganglion cells separated by fiber tracts. The loose fibroelastic connective-tissue sheath is partly torn away from the ganglion.

This connective tissue covers the ganglion and extensions from it continue internally, partly separating masses of cytons and fibers. The cytons are large spherical cells of different diameters. Within each is a large nucleus which usually contains a nucleolus. Around each cyton may be seen a row of nuclei, indicative of a sheath of indifferent cells—the so-called satellite cells, which are homologous with neurolemma cells. Elsewhere are clumps of fibers, usually with myelin and neurolemma. The single process from the cyton is rarely visible in a section. A short distance away from the cyton it divides into a peripheral and central process. The peripheral

branch is regarded as a specialized dendrite. The central process is regarded as an axon which extends from the ganglion into the dorsal horn of gray matter on that side of the spinal cord.

Outside the cord and brain are several sympathetic ganglia of such size and uniformity of location that they have received anatomical names. But in addition to these which can be located by dissection, there are a great many small ganglia that can be seen only in microscopic preparations. The cells of sympathetic ganglia have long axons and dendrites which in some cases extend out beyond the capsule cells to form connections with adjacent cells. In other cases the dendrites are entirely within the capsule. Connective tissue surrounds the ganglion and penetrates among the fibers and cells. Myelin is not usually found surrounding the sympathetic fibers, but a neurolemma sheath does occur.

DEGENERATION OF NERVES.

Degeneration changes appear soon after a peripheral nerve is severed. Before this occurs, however, there is an almost immediate chemical change which extends in both directions from the lesion. This effect is known as traumatic degeneration and extends centrally to reach the cytons of the neurons involved. The Nissl substance disintegrates and disappears. Although the cytons are affected by the lesion they possess the power of recovery. This recovery phase soon follows and extends out along the nerve fibers as far as the lesion. But the portion of the nerve distal to the lesion does not recover and disintegration of the myelin and axon progresses outward from the lesion. A great increase in neurolemma nuclei has been observed to accompany these changes. In about three days after the lesion is made, the nerve no longer conducts and the structure innervated by this particular nerve is no longer served. If the nerve supplies certain muscles, these will be paralyzed, since motor impulses are no longer being sent to them. Paralysis will be permanent unless the nerve regenerates.

REGENERATION OF NERVES.

If, soon after the lesion is made, the cut end of the peripheral portion of the nerve is brought into contact with the cut end of the central portion and kept in this position, then the neurolemma cells which absorbed the broken-down axon and myelin material will form protoplasmic bands. These protoplasmic bands serve as

tracks along which the sprouting ends of the cut central portions of the axon will grow, and the developing new axons will find their proper terminations. It is very important to have no scar tissue formed at the lesion where the cut ends of the injured nerve are brought together. Regeneration is more rapid in young animals than in old and also more rapid in warm-blooded types. Degeneration proceeds centrally in some cases, and involves cytons and dendrites. No regeneration of such cells takes place. Nor is there complete regeneration in the central nervous system. The phenomenon of Wallerian degeneration has been of great value in aiding the determination of the central origin and peripheral termination of groups of nerve fibers. An experimental lesion is made involving a small area in the spinal cord. Proper time for degeneration of the nerve fibers concerned is permitted to elapse. The animal is then killed. The cord is removed and serial sections made following the proper technique devised for the purpose. Disintegrating fibers and cytons have a distinguishing appearance and enable the investigator to follow the route of the degeneration and thus determine the origin of the fibers occupying the region of the cord involved in the experimental lesion.

THE BRAIN AND SPINAL CORD.

Both the brain and spinal cord are suspended within the cartilaginous or bony capsule by several connective-tissue membranes. The outermost, the dura mater, is a thick fibroelastic connective tissue attached to the bony or cartilaginous capsule. A narrow cleft, the subdural space, contains fluid and separates the dura from an innermost membrane, the arachnoid, a thin connective-tissue membrane. Connecting strands from the arachnoid to the dura mater divide the subdural space into compartments, others join it to an innermost membrane, the pia mater, immediately surrounding the brain or cord. The spaces of the arachnoid are filled with fluid, the cerebrospinal fluid, a term applied also to the fluid within the ventricles of the brain and the canal of the spinal cord.

The brain like the spinal cord has a region of gray matter occupied by cytons, nerve processes, and neuroglia; and a white matter occupied by the processes and neuroglia only. Among the lower forms the cytons are fewer. The cerebrum and cerebellum of mammalian brains may be recognized by the type and arrangement of cells; in the cerebral cortex the pyramidal cells are characteristic, and in the cerebellar region the Purkinje cells are diagnostic features.

Structure of the Spinal Cord. — The gray matter in the cerebrum and cerebellum is external and covers the white matter. In the spinal cord the gray matter is internal and more or less surrounded by white matter. The cord is almost divided into two halves by a deep median dorsal septum and a shallower, wider, median ventral fissure. However, the two halves are connected by a white and gray commissure. The gray matter resembles the letter "X" or letter "H." The narrower dorsal horns of gray matter extend almost to the periphery. (Fig. 63.) The ventral horns are much wider
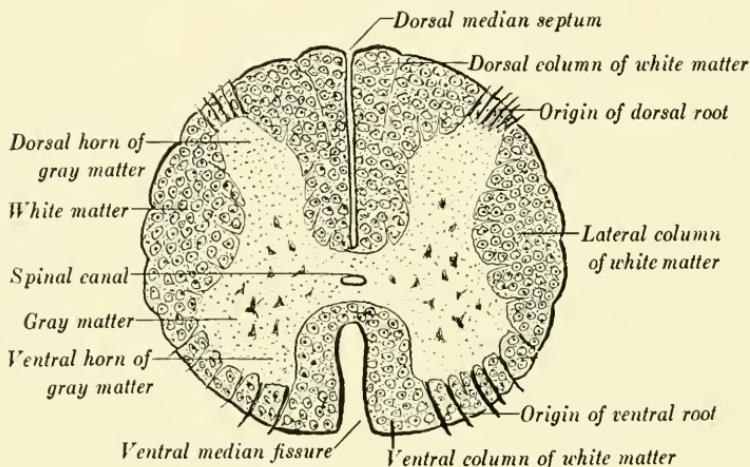
Fig. 63. — Diagram showing regions of the spinal cord.

and do not extend to the ventro-lateral surface. The dorsal median septum extends down to the gray commissure, and the latter contains the canal of the spinal cord which is lined by ependymal cells. Ventral to the gray commissure is a white commissure. The white matter between the dorsal septum and the gray horn adjacent to it is known as the dorsal funiculus or column of white matter. Ventral and lateral to the dorsal horns on either side is the ventro-lateral column or funiculus. The latter white matter may be subdivided into a ventral column between the ventral fissure and the gray matter of the ventral horn adjacent to it. The white matter dorsal and lateral to this is known as the lateral column of each side. The white matter consists of myelinated and unmyelinated axons, and neuroglia but no neurolemma. The surface of the cord is immediately covered with pia mater and this extends into the white matter as short septa in places. Studies of various kinds

have shown that the axons of the white matter occur in strands of well-defined location. The axons of the dorsal columns carry sensory impulses up the cord; those of the ventral columns carry motor impulses down the cord; and the lateral columns are subdivided into tracts, some of which are sensory and some motor. The gray matter of the cord consists of neuroglia; axons entering from the columns; entire short neurons; cytons, dendrites, and the initial portions of axons which extend further beyond the gray matter into the ventral roots of spinal nerves and on out into such nerves. The neurons of the gray matter are multipolar in type and have already been described. Axons of some gray matter neurons extend out into the white matter and are incorporated as part of a white column.

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See Appendix for general text references.

CHAPTER VII.

THE VASCULAR SYSTEM.

In various regions of early embryos, groups of mesenchyme cells begin the development of the vascular system. The central cells of such an area become rounded and separated by a fluid intercellular plasma. The peripheral cells of these regions unite to form an endothelial tube enclosing the free primitive blood cells and the plasma. The thin walls about these spaces are interconnected with others, so that gradually a network of endothelial-walled tubes forms the first capillary system. Some of the early capillaries develop into arteries, others into veins, and a tubular part is later differentiated into the heart. In the development of an artery and a vein, not only is an enlargement of the tube brought about, but there is also an addition of fibroelastic connective tissue and smooth muscle organized in sheaths about the endothelial lining.

THE CAPILLARIES.

These narrow, delicate, endothelial-walled tubes form a vast network in the connective tissue throughout the body. The diameter varies from a minimum of slightly less than the diameter of an
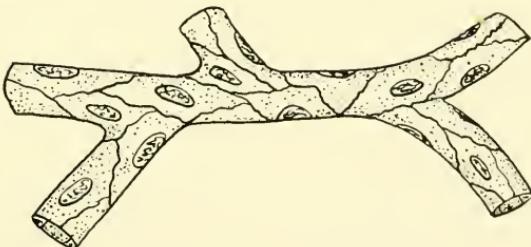
FIG. 64.—Diagram of part of a capillary network, showing endothelial cells

erythrocyte to a diameter several times this. (Figs. 64 and 65.) In cross-sections of very small capillaries only one or two endothelial cells form the wall, but in larger tubes a number of cells are present. The boundaries of the endothelial cells show as irregular black lines after silver nitrate treatment. The cells are elongated in the direc-

8

tion of the flow in the lumen and each cell has a nucleus in the
center of a clear cytoplasm. In fixed preparations, the capillaries
contract and in cross-sections the nuclei appear to protrude into
the lumen, a condition not true of living capillaries.

Some investigators conclude that capillaries are intrinsically
contractile, and others believe that the contractility is due to
certain flat irregular cells, similar to reticular cells, which lie outside
the capillaries but in close contact with the wall.

It is well known that plasma and leukocytes from the blood stream
pass out into tissue spaces through the thin capillary cell and also
that wastes in fluid form pass from tissue spaces into the stream
within the capillary. Some investigators claim that the exchange
is facilitated by minute openings where adjacent cells meet, but
in general the endothelial wall appears quite unbroken. The
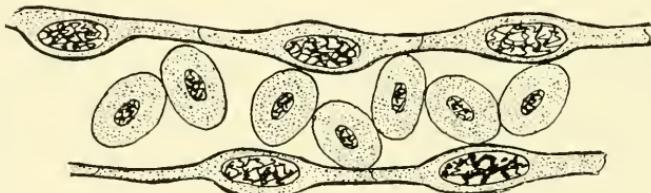
Fig. 65.—Sectional view of a capillary in the gill of a dogfish and erythrocytes within it.

chemistry and physics of passage of fluids and white cells from the
capillary and back into it are incompletely known.

Usually the blood-vascular system of a vertebrate is regarded as
a closed system in that the erythrocytes do not normally leave it.
In every other respect it is an open system. There is a constant
passage of food compounds from the intestinal tract into capillaries
and lymphatics in the villi where they lie near the epithelium.
Oxygen diffuses through the wall of the air sacs of the lung and is
taken up by erythrocytes in capillaries adjacent to the air sac wall.
Wastes of metabolism also pass from tissue juice into capillaries.

The arrangement of the capillary network is determined to a
considerable degree by the disposition of the cells or tissue organiza-
tion supplied by the capillaries. Capillaries supplying skeletal
muscles are long tubules between adjacent muscle fibers and are
connected laterally. In the kidney there is a rich capillary network
between adjacent uriniferous tubules. In each villus of the small
intestine there is a basketwork of capillaries in the stroma just
within the epithelial wall. Secretory vesicles of the thyroid gland

are enclosed in delicate connective tissue in which lies a network of capillaries. The richness of the capillary network is related to the functional activity of the organ supplied. Where great functional activity of the organ is constant, the meshwork is close and the capillaries are large. A good illustration of this is seen in the capillary network about the alveoli of the lung and the tubules of the kidney. The capillary system of the liver and spleen is atypical and will be described when considering these organs.

Healing of tissue lesions may involve development of new capillaries and of small arterioles and venules. In case such structures form, they first appear as buds from existing capillaries.

An examination of the circulation in the web between the toes of the frog's foot reveals a rapidly flowing stream of blood cells in the small arterial branches and a much slower streaming in the network of capillaries. Erythrocytes are carried along like leaves in a stream, some having to bend in making a turn from one channel into another. The slow flow through capillaries permits diffusion of food and oxygen out into the tissue spaces and a return of organic wastes to the blood stream from the tissues. As the capillaries unite to form small veins, the speed of the current increases again, but is not as rapid as in the corresponding arteries.

THE ARTERIES.

The transition from capillaries to arteries is marked by the gradual appearance of smooth muscle and fibroelastic connective tissue. (Fig. 66.) In general, three coats are usually indicated in a study of the wall of an artery: an inner, tunica intima; a middle, tunica media; and an external, tunica externa. The intima is composed of the lining endothelium with a slight amount of fibroelastic connective tissue. The media is characterized by smooth muscle associated with varying amounts of fibroelastic connective tissue. The adventitia is chiefly fibroelastic connective tissue. Arteries are usually divided into three groups on the basis of size and composition of the media. The large or elastic arteries include the aorta, pulmonary arteries, carotids, and a few others with a similar structure. The medium-sized, or muscular, arteries include other arteries named by anatomists; these vessels agree in structure, although they vary in size. To the small arteries belong a great number that have no special names. Two more groups might be added, namely, arterioles and precapillary arterioles. Although the composition of

all these groups varies, there is a gradation of the type of construction all along the line, as can be understood best by studying the smallest arteries first.

Small Arteries.—The smallest branches adjacent to capillaries have a small amount of connective tissue supporting isolated smooth muscle cells outside the endothelium. These are pre-capillary arterioles. They are branches of larger arterioles which have a definite smooth muscle sheath surrounded by a sheath of fibroelastic tissue. The small arteries have thick walls in comparison with the size of the lumen. The intima has a thin subendothelial layer of fibro-

1 *Capillary*
2
3 } *Arterioles*
4
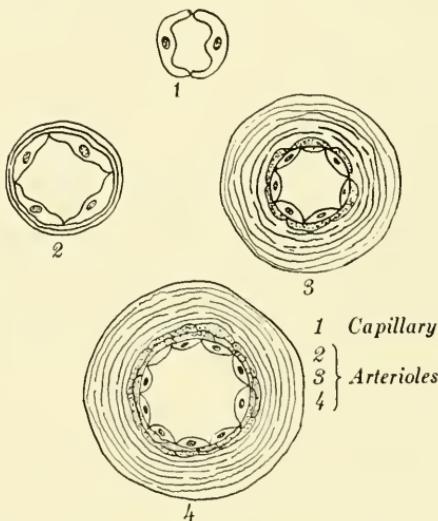
Fig. 66.—Diagram of small arterioles and a capillary.

elastic connective tissue separated from the media by an internal elastic lamina, a membrane of elastic fibers. The media is composed mainly of smooth muscle cells interspersed with fibroelastic connective tissue and is separated from the adventitia by an external elastic lamina which resembles the internal membrane. (Fig. 67.) The adventitia is composed of fibroelastic connective tissue with a very few smooth muscle cells. It is usually not as thick as the media but much thicker than the intima. With the addition of the muscle tissue the vessels take an active part in distributing blood by rhythmic contractions or peristaltic action. These small arteries grade into medium-sized arteries.

Medium-sized or Muscular Arteries.—These are larger than the preceding vessels and the predominant tissue of the media is smooth

muscle. In the larger branches the proportion of elastic fibers in the media increases. The intima is often poorly preserved, the endothelial cells being indicated by their nuclei which protrude into the lumen. The subendothelial connective tissue is generally inconspicuous but is better seen in the larger vessels of this class. The internal elastic membrane appears as a clear, wavy line and the thick media shows numerous layers of smooth muscle separated by connective tissue. In larger vessels, elastic fibers appear more prominently in the media. The adventitia is as thick as the media or may be thicker, with elastic fibers becoming more numerous toward the media.
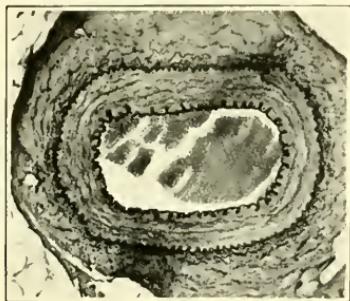
FIG. 67.—A photograph of a small artery of the cat, showing internal and external elastic membranes outlining the muscular and elastic media.
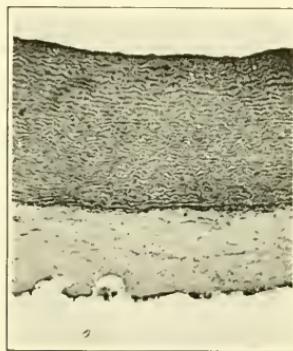
FIG. 68.—Aorta of the dog, fixed in Bouin's fluid and stained with hematoxylin and eosin, showing the heavy elastic media and fibrous adventitia.

Large or Elastic Arteries.—These vessels, such as the aorta and the pulmonary arteries, have a relatively thick wall which appears thin in view of the large size of the lumen. When compared with a medium-sized artery the muscle has decreased in amount and there is a greater supply of elastic fibers. The intima is thicker due to the presence of more subendothelial connective tissue. The internal elastic lamina is not evident and in the media are many layers of fenestrated elastic membranes which are seen best when orcein or resorcin fuchsin is used as a stain. (Fig. 68.) Alternating with the elastic tissue are smooth muscle and collagenous fibers. The media appears to be very much thicker than the adventitia, which is composed of fibroelastic tissue with few, if any, smooth muscle cells. At the origin of the aorta and pul-

monary arteries from the heart, the walls may be composed mainly
of cardiac muscle. The fibroelastic tissue forming the outer coat of
the adventitia in all vessels merges with the connective tissue
supporting the vessel.

The main function of arteries is to conduct blood away from the
heart. The contraction of ventricles forces blood into the great
arteries originating from them, and these vessels already filled with
blood are distended by the added supply. When the force of ven-
tricular contraction is spent, the fluid tends to return to the ven-
tricles but is prevented by the closing of the valves at their entrance.
Then the distended elastic walls contract as the stretched elastic
tissue recoils. The blood is thus sent on into the medium-sized
arteries where the smooth muscle takes a part and active peristaltic
waves propel the blood onward.

THE VEINS.

Passing from a capillary toward the heart, there are postcapillary
veins, venules, small veins, medium-sized veins, and large veins,
showing varying structural additions and modifications. Outside
the endothelial lining smooth muscle and fibroelastic connective
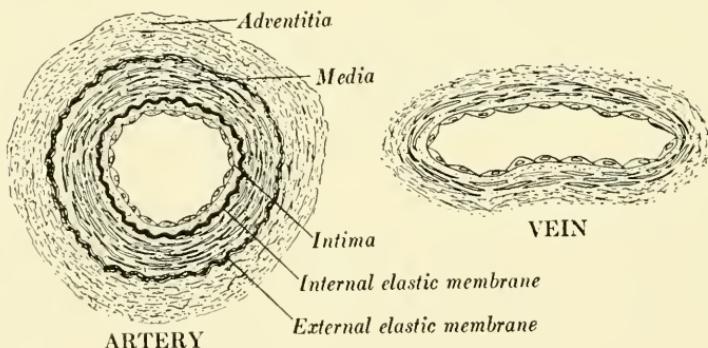
Fig. 69.—Diagram of small artery and vein.

tissue form the added tissue in the wall of veins, but there is such
a great variation in structure that each vein must be studied for its
particular type of organization. On the whole, however, it can be
said that the wall of any vein is thinner than that of its accompany-
ing artery, and consequently veins often appear collapsed in micro-
scopic preparations. (Fig. 69.) Elastic laminæ are not usually
present and in many cases it is difficult to distinguish all three

coats. The structure seems to be influenced by gravitational diffi-
culties, so there appears to be more muscle in veins of the extremities
than in those nearer the heart.

Veins differ from arteries in that all except very small veins
possess valves which are pocket-like extensions of the intima with
a core of subendothelial connective tissue. (Fig. 70.) Such valves

Valve

Intima

Media

Adventitia
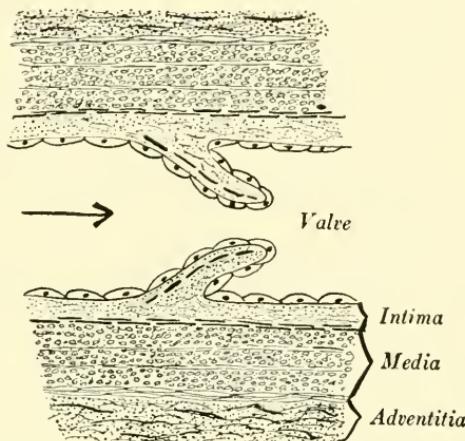
Fig. 70. —Diagram of a vein showing a valve.

open toward the heart so that a backward flow of blood is prevented.
In the connective tissue that supports an artery there is usually a
companion vein which has a greater volume and usually appears
collapsed in microscopic preparations. In the adventitia of arteries
and veins, but not in arterioles and venules, there occur blood-
vessels called vasa vasorum, which provide a capillary network for
the wall tissue of the vessel. Similarly a nerve supply, known as
the nervi vasorum, is present. The outer coat of the larger arteries
and veins supports a system of small lymph vessels also.

THE LYMPH VESSELS.

In general, these vessels resemble veins in structure but have
thinner walls and more valves. Lymph capillaries are larger than
blood capillaries, are thin walled, and not commonly seen in ordinary
preparations. In the largest lymphatics, three coats may be differ-
entiated. When preserved uncollapsed, the smaller ones appear
as endothelial-lined spaces in loose fibroelastic connective tissues.
These vessels are larger than the veins whose distribution they

parallel, but their walls of connective tissue and scattered smooth
muscle cells collapse and are not usually readily distinguished in
preparations.

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See Appendix for general references.

CHAPTER VIII.

THE LYMPHATIC SYSTEM.

The lymph vessels develop in a manner similar to, if not as outgrowths of the embryonic venous system. In either case a system of closed endothelial tubes is formed and at intervals develop valves, as in the case of the veins. According to Drinker and Field, "the lymph capillaries are complete vessels, whose content is identical with the fluid outside them. The barrier presented by their walls is extremely slight. It serves to guide their contents into heavier walled channels from which escape is difficult, and by means of which return to the blood is accomplished. Capillary lymph and tissue fluid are thus considered to exist in a common reservoir, and to this the blood capillaries make addition of fluid and by resorption withdraw it." In the formation of the larger vessels, mesenchyme cells surrounding the endothelium develop a connective-tissue wall in which some smooth muscle also differentiates.

The smallest blindly ending divisions of these vessels, the lymph capillaries, collect fluid from spaces between the fibers and cells of connective tissue. This fluid passes from the capillaries into the smallest lymph vessels, thence into larger and larger branches, which ultimately join the venous system in the region of the heart. Lymph resembles the blood in having a plasma containing free cells, the lymphocytes, but lacks erythrocytes and granulocytes.

At intervals along the lymphatic system, active mesenchymal elements develop into lymphoid organs in which lymphocytes are produced and are added to the colorless, fluid lymph. Lymphoid organizations are especially well developed in mammals where lymphocyte production is carried on apart from the production of other myeloid cells. Among birds and reptiles, and in the amphibians to a lesser extent, lymphocytes may be formed in lymphoid tissue scattered in various parts of the body, but the spleen is the major lymph organ in these forms. In mammals, particularly, there is a variety of such organizations, all characterized by the presence of one or more lymph nodules.

THE LYMPH NODULES.

These are oval or round, densely packed masses of lymphocytes supported in a reticulum of connective tissue. They vary in size and are commonly located singly in the subepithelial connective tissue along the extent of the digestive and respiratory tract. Each nodule has a connective-tissue framework within whose meshes are lymphocytes in various stages of development. The central, more diffuse region of a nodule, known as the germinal center, is occupied by the less differentiated cells. This is a region of mitotic activity, from which new cells become differentiated and are pushed toward the periphery.

Agminated Lymph Nodules.—There are groups of nodules located along side one another in the subepithelial connective tissue of the digestive tract. In the ileum of mammals, near its junction with the large intestine, are groupings of many nodules, called Peyer's patches. (Fig. 71.) Some of these extend through the epithelium
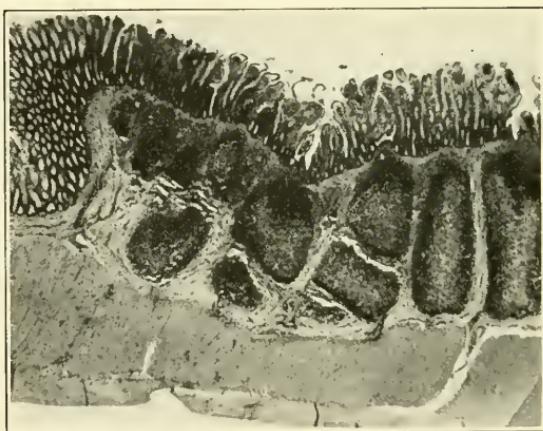
FIG. 71.—A group of nodules (Peyer's patch) below the epithelium of the ileum of the dog.

into the lumen of the intestine and also into the submucosal coat. Another collection of nodules ocurs in the connective tissue adjoining the epithelium of the cecum and vermiform appendix.

Tonsils.— In mammals, several prominent aggregations of lymph nodules, known as tonsils, are found in the pharynx. They differ in location but have a similar organization. A group composing the faucial or palatine tonsils is located on each side of the pharyngeal cavity between the pillars of the fauces. The lingual tonsil is located

beneath the non-papilliated epithelium of the upper surface of the tongue posterior to the foramen cecum. Another group, composing the pharyngeal tonsil, lies just below the epithelium in the upper posterior face of the pharynx near the entrance of the nasal passages. Similar masses of lymphoid tissue at the entrance of each Eustachian tube form the tubal tonsils. In all these structures, groups of nodules lie below a stratified epithelial membrane. (Fig. 72.) Scattered crypts, or pits, may occur in the epithelium and extend down into the tonsil tissue. These crypts are lined with stratified squamous epithelium which rests upon a stroma of
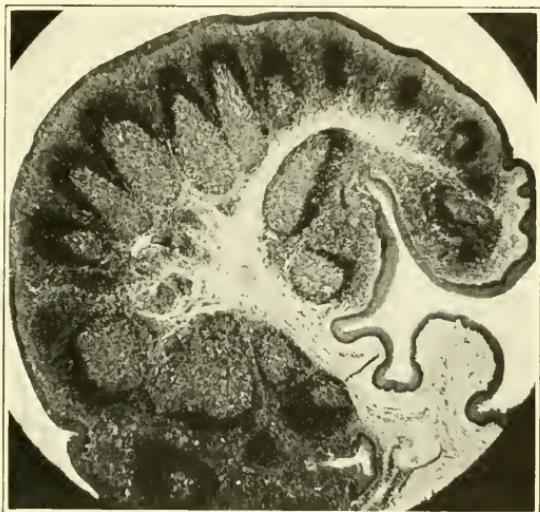
Fig. 72.—Tonsil of the dog. The nodules are closely packed below a stratified squamous epithelium.

fibroelastic connective tissue continuous with similar tissue forming the coarse framework for the nodules. The lymph nodules are sometimes clearly separated, but may have diffuse lymphoid tissue connecting them. A fine network of reticular connective tissue extends through the nodules and between them. Below the lymphoid mass is a dense fibroelastic connective tissue which merges with similar tissue associated with the skeletal muscles of the neck. Tonsil tissue gives rise to a supply of lymphocytes, many of which may be lost by passing through the epithelial membrane; others may enter capillaries associated with the reticulum. The tonsils commonly atrophy but may become hypertrophied under conditions of infection. Their removal is often followed by regeneration of lymphoid tissue in the same locality.

THE LYMPH NODES.

The lymphoid structures so far studied are associated with the lymph capillaries, *i. e.*, they are situated near the beginning of lymph vessels. A more elaborate organ, the lymph node, found widely distributed in mammals, is an oval or bean-shaped gland interpolated along the course of larger lymph vessels. Lymph enters the node by one or more afferent lymph vessels and leaves by one or more efferent lymphatics. Lymph nodes vary in size from a few millimeters in length to 2 centimeters or more. They are also indefinite in number, since they may degenerate or new nodes may form in the loose fibroelastic connective tissue of certain
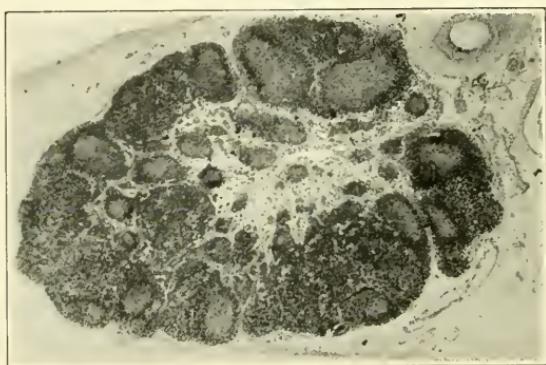
Fig. 73.—Mesenteric lymph node of the gray squirrel, showing dense cortical region and lighter medulla. The connective tissue of the capsule extends into the medulla at the hilum and forms trabeculæ in the cortex.

regions. Lymph nodes are prominent in the mesentery near the junction of the small with the large intestine and also in the connective tissue in the region of the axilla and the groin. (Fig. 73.)

Each node is invested with a capsule of fibroelastic connective tissue in which smooth muscle cells are usually scattered. The capsular tissue is more evident at the hilum, or concave depression, where the efferent lymph vessel, an artery, vein, and nerves are connected with the node. In examinations of stained preparations of mammalian nodes, two regions, an outer cortex and an inner medulla, usually stand out clearly. (Fig. 74.)

Cortex.—Incomplete partitions, or trabeculæ, of fibroelastic connective tissue extend from the capsule into the interior, thus dividing the cortex roughly into compartments. In these compartments are

lymph nodules, varying in shape, though usually composed of oval or pyriform masses of lymphoid cells. Extending from the coarser connective-tissue framework is a finer network of reticular cells and fibers in whose meshes the lymphoid cells are held. The reticulum extends between the nodules and trabeculæ and capsule, forming cortical sinuses. Afferent lymph vessels entering the capsules break into capillaries connecting with these sinuses through which the inflowing lymph passes into the interior. The lymphocytes produced in the nodules move through the reticulum into the cortical lymph sinuses through which lymph is slowly passing toward the medulla. The size of the germinal centers varies with the degree of mitotic activity in them, the active nodules having more marked
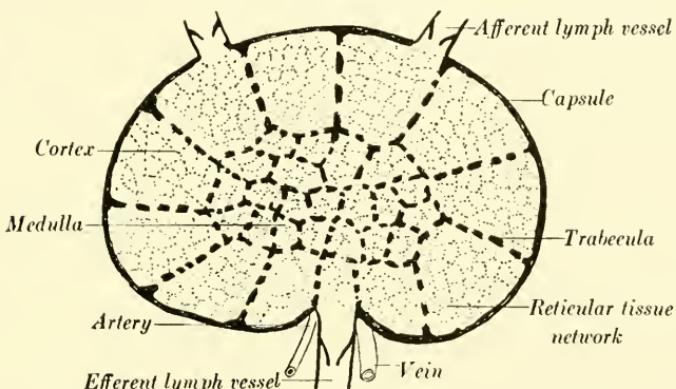
FIG. 74.—Diagram of a connective tissue and reticular tissue skeleton of a lymph node.

germinal centers. Cells of the sinus walls become macrophages and remove foreign elements from the lymph as it filters through. (Fig. 75.)

Medulla.—The internal ends of the connective-tissue trabeculæ form a network of coarse fibers between which extends the reticulum of argyrophil fibers and reticular cells. Clustered along the coarser connective-tissue network are rod-like masses of lymphoid cells known as medullary cords. Between them are interconnecting spaces, the medullary sinuses, with which the cortical sinuses are continuous. The walls of the sinuses in the cortex and medulla are formed by reticular and endothelial cells which, in case of invasion by foreign substances, such as bacteria, become active phagocytic cells, or macrophages. There is a slow distribution or circulation of lymph from the lymph vessels at the cortex through

the sinuses into the efferent lymph vessels at the hilum. During this passage through the sinuses a number of lymphocytes are added so that lymph leaving the node is richer in cells than when it entered.

Three varieties of lymphocytes (small, medium, and large) have been distinguished in the node, but by far the greater number are the small variety. When mitotic activity is at its height the germinal center is filled with lymphocytes of the small variety similar to those in the peripheral portion of the nodule. Then proliferation ceases for a time and during this resting period the nodule has a uniform appearance throughout. Even isolated nodules have periods of rest and activity.
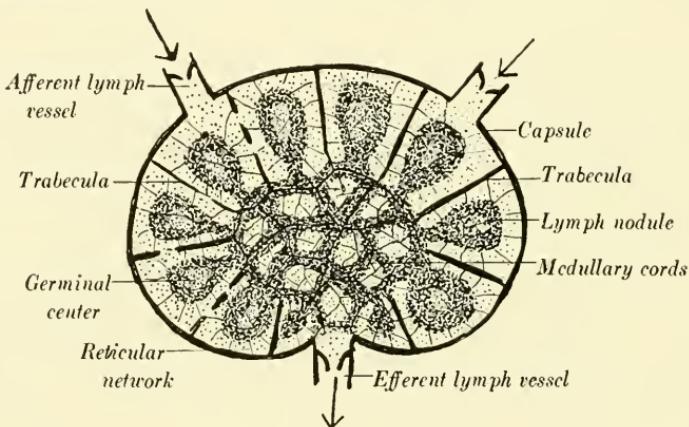
Fig. 75.—Diagram showing structural plan of a lymph node. The cortex is the outer zone containing lymph nodules. The medulla is the central region with cords of lymphocytes.

The artery entering at the hilum branches throughout the fibro-elastic connective tissue skeleton, breaking up into capillaries which connect with venules and the latter eventually with the vein or veins which leave from the hilum. Small arterioles in trabeculæ give rise to a capillary net which surrounds the nodules. So far as erythrocytes are concerned, they remain within the vascular system in the node, i. e., there are normally no free red blood cells in the sinuses. Trabeculæ appear to be better developed in peripheral lymph nodes, such as those in the axilla or groin. Nodes within the body cavity have relatively greater medullary regions. Trabeculæ of small nodes are difficult to locate, and in some nodes the cortex is massed chiefly at one end while the medulla is concentrated at the other.

Lower vertebrates do not show such marked lymphoid organization. In the frog there are scattered and very small oval or spherical bodies composed of accumulations of lymphocytes in a network of connective tissue surrounded by a fibroelastic capsule. Fine trabeculæ may divide the structure, but medulla and cortex are not differentiated. Presumably the lymphocytes produced here enter lymph capillaries supported in the connective tissue of the organ.

THE HEMOLYMPH GLAND.

Although the lymph node appears limited to mammals, somewhat modified structures, called hemolymph nodes or glands, are found in mammals and birds. In these lymphoid organizations, which structurally resemble the lymph nodes, blood instead of lymph is distributed in the cortex from arterial capillaries and filters through sinuses to be collected by efferent veins. Lymph vessels are limited to the scanty trabecular tissue.

THE SPLEEN.

The spleen is the largest single lymph gland. It is similar to hemal nodes, which are often called accessory spleens, in being associated with the blood-vascular system instead of lymphatics. Its shape and location varies in different vertebrates; among the fishes it may be an irregular mass closely associated with the stomach and intestinal wall; in others, as in the frog, it occurs as a spherical mass in the mesentery supporting the intestine; among the mammals it is a flattened structure near the stomach.

The spleen is surrounded with a capsule of fibroelastic connective tissue and smooth muscle covered with mesothelium, but there is no division into a distinct cortex and medulla. Trabeculæ of connective tissue and smooth muscle extend from the capsule to form a coarse internal framework. A reticulum like that in the nodes extends between the trabeculæ and forms the inner support for the lymphoid tissue. The larger arteries and veins supplying the spleen are carried by the trabeculæ.

Examination of slices of fresh mammalian or avian spleen reveals small, whitish masses, the white pulp, distributed throughout a red tissue. Each white pulp mass is a splenic nodule (splenic corpuscle or Malpighian corpuscle) which is composed of a mass of lymphocytes enmeshed in a reticular network, and is comparable to a solitary lymph nodule or to a cortical nodule of a lymph node.

(Fig. 76.) Within each is one or more small arteries, usually eccentric; a feature which serves to identify spleens so organized. The red pulp is composed of a reticular network, diffuse lymphoid tissue, and sinusoids filled with blood.
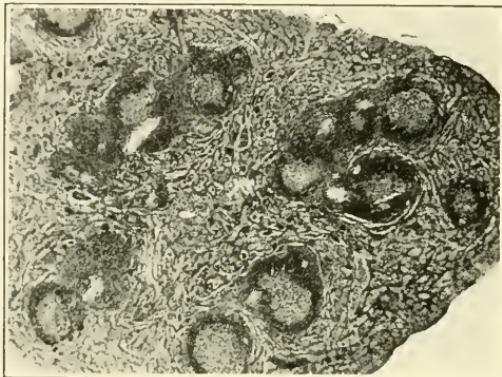
FIG. 76.—Spleen of the woodchuck, showing the large splenic corpuscles scattered through the diffuse red pulp. The nodules have light germinal centers and arterial branches appear in their peripheral portion as small, open, white areas.

The microscopic structure of a mammalian spleen is clarified by an understanding of the distribution of blood vessels in it. The main splenic artery and splenic vein enter at the hilum and each
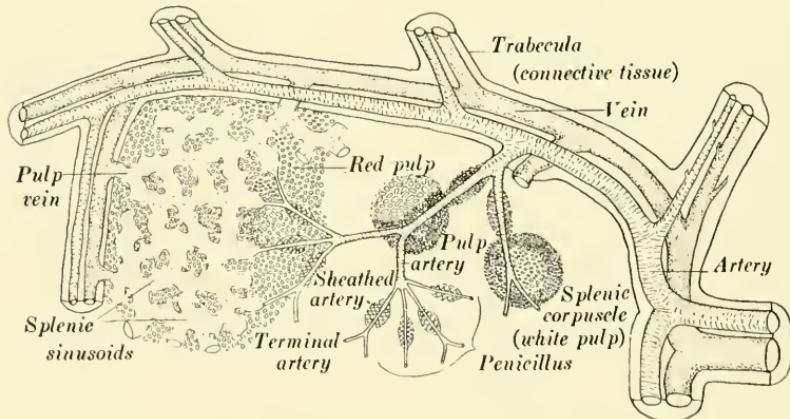
FIG. 77.—Diagram showing scheme of circulation in the spleen.

vessel divides into branches which are supported by the branching trabeculæ. As the trabeculæ divide, each supports a branch of an artery and an accompanying vein. Small arterial branches leave the trabecular tissue and enter the lymphoid tissue. (Fig. 77.)

The adventitia of such arteries has loose fibroelastic connective tissue in which lymphocytes become aggregated into rod-like or oval masses, the splenic nodules, or Malpighian corpuscles. The branches of these small arteries emerge into the red pulp, where they are known as pulp arteries. Each pulp artery divides into a group of short vessels, the penicilli, which have a pulp, a sheathed, and a terminal region. The tunica media of the first division is rich in smooth muscle and is embedded in red pulp; the sheathed portion is devoid of muscle and has a narrow lumen surrounded by connective tissue infiltrated with the lymphocytes and connected with the reticulum of the red pulp; the terminal portion is an endothelial tube which unites with the peculiar sinusoids in the red pulp.
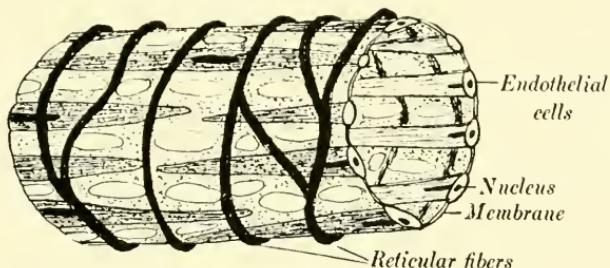
FIG. 78.—Diagram of part of a splenic sinusoid which consists of long endothelial cells, a non-cellular membrane, and encircling a network of reticular fibers. Openings are indicated from the lumen of the sinusoid through the membrane. (Redrawn from Braus, in Bailey's Histology, Williams & Wilkins Company.)

The sinusoids (Fig. 78) have a wider lumen than ordinary capillaries, and their elongated endothelial cells are separated from each other at intervals and are associated with the reticular cells which form a network through the red pulp. At intervals, fusiform cells and reticular fibers encircle the endothelial cells. The many slit-like spaces in the walls of the sinusoids communicate with the loosely organized lymphoid tissue of the red pulp. The sinusoids join complete endothelial-walled venous capillaries, which continue into venules and veins carrying the blood back into the trabeculæ and out of the spleen. (Fig. 79.)

The sheathed portions of the penicilli where the lumen is small slow down the blood issuing from the small arteries of the splenic sinusoids. The sheathed penicilli may act as valves, preventing backward flow of blood. In the red pulp of the spleen and of hemal nodes, definite openings in the blood-vascular system

9

occur. Cells in the blood stream can pass through the slits in the sinusoid wall into the red pulp proper and cells in the red pulp can pass into the blood stream by way of the sinusoid. In the reticulum and diffuse lymphoid tissue of the red pulp there are the same red and white cells found in the circulating blood. The lymphocytes produced in the nodules enter the reticulum of the red pulp and thus may pass directly into the blood stream. Also in the red pulp are numerous giant cells, macrophages presumably derived from reticular cells in which phagocytic activity is pronounced. Foreign elements and disintegrating blood cells are ingested by these cells.
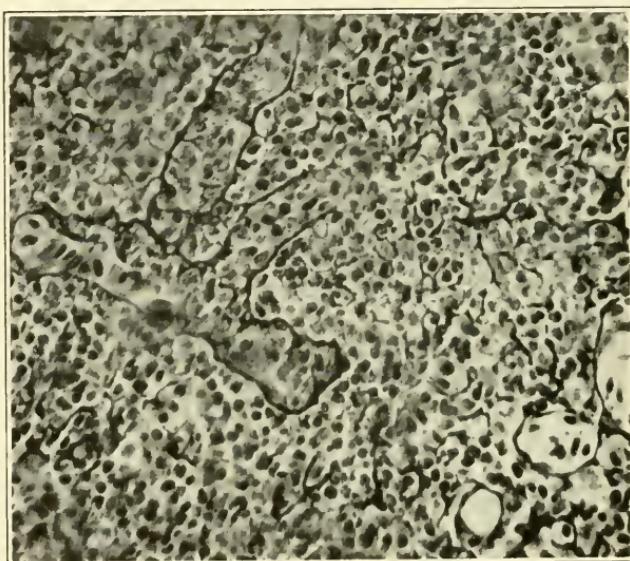
Fig. 79.—Photograph of spleen of Necturus, showing the vascular distribution; the vessel on the left is shown with several branches opening out into sinusoids. Mallory stain.

In certain pathological conditions so many red cells are disintegrating and so many are being phagocytized that the red pulp acquires a brown color. The endothelial cells also appear capable of phagocytic activity and ameboid movement.

In embryonic mammals and in lower vertebrates the spleen is hemopoietic, and lymphocyte production is not so clearly localized in nodules. The structure remains fundamentally the same, except that nodules are no longer clearly differentiated, lymphocytes and erythrocytes both being produced in a common reticulum, as already observed in the chapter on blood. Although erythrocytes

are produced in the spleen of the embryo mammal, this power is later transferred to the red marrow of bones. However, in certain pathological conditions, erythroblasts and the three types of myelocytes can be found in the spleen, showing that in such cases production of erythrocytes and granulocytes can again occur here.

A recent review of lymphatics by Drinker and Fields corroborates an early account of Mollier concerning the open nature of this portion of the vascular system—open to the extent that blood with its cells can freely though sluggishly pass into diffuse lymphoid tissue of the red pulp. The splenic sinusoids join complete venous capillaries, which continue into small venules projecting through the red pulp to enter the trabeculae. In the spleen, blood can leave the vessels and mingle with surrounding cells without clotting. Blood-pressure is very low here, and periodic pulsations of the entire spleen have been observed; a periodic contraction forcing blood from the pulp into the sinuses and veins is followed by a dilatation. The smooth muscle in the capsule and trabeculae effects this contraction. The connective tissue of the capsule limits the dilatation or the accumulation of too much blood as the contraction of the smooth muscle imparts a gentle pressure forcing the blood back into the veins. The absence of cellular injury when blood enters the red pulp from the sinusoids apparently accounts for the failure of clotting of such extravascular blood.

THE THYMUS.

The thymus occurs throughout the vertebrate groups as a variable structure beginning as invaginations of the epithelium of the gill clefts that invade the underlying mesenchyme. The thyroid and parathyroid anlages are similarly derived. In some fishes the epithelial tissue appears predominant, but in most forms the associated mesenchymal elements develop a lymphoid tissue about the epithelial elements. This lymphoid tissue becomes more prominent among reptiles, birds and mammals. The epithelial tissue is usually cut off from the gill pouches during embryonic development. In the lower forms the thymus may remain as a varying number of small buds associated with the gill pouches from which they were derived. In the mammals a common lobulated mass is usually formed and lies along the lower part of the esophagus and extends into the thorax over the pericardium.

In general, a thin-walled capsule of fibroelastic connective tissue

surrounds the entire gland and extensions of it divide the gland into small lobules. (Fig. 80.) Under the microscope each lobule appears to be a mass of lymphoid tissue with a darkly staining peripheral cortex of more dense lymphoid cells and a central diffuse medulla. The medullary portion of adjacent lobules connect with each other. The lymphoid cells of the cortex are very similar to small lymphocytes. At the border between cortex and medulla there is an abrupt change to the diffuse distribution of cells in the medulla. Each lobule has a network of reticular cells and fibers continuous with the coarse framework of loose fibroelastic connective tissue. The lymphoid cells are considered by some not to be
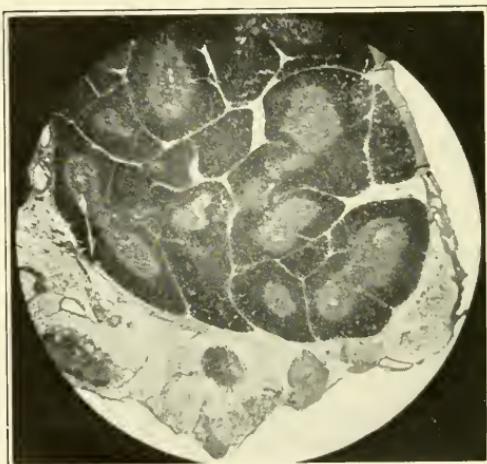
FIG. 80.—Photograph of a section through a portion of a child's thymus gland. Several lobules are shown with cortex and medulla. Note connection of the medullary portions of some adjacent lobules.

typical lymphocytes, and as derivatives from embryonic endoderm are differentiated as thymocytes. However, their appearance, their ameboid motion, and other reactions are so similar that the usual view is that they are lymphocytes derived from mesenchyme elements that migrated into this region.

The reticular cells forming a network through the tissue are considered as derivatives of mesoderm into which the epithelial thymic invagination extended, and as derivatives of endodermal epithelium. From whatever source, these reticular cells of the thymus appear very similar to those found in other locations. Fusiform cells, called myoid cells, have striated fibrils and occur commonly in the thymus of a number of forms, but not in mammals

after birth. Much remains to be done to determine the exact nature of the elements composing this organ.

In the medulla of many lobules, fusiform cells are concentrically arranged to form smaller cell masses known as Hassal's corpuscles. In the center of some there may be deposits of calcium or small cysts surrounded by flattened cells. The significance of these bodies is debated.

The thymus differs from other lymph organs in that it has no nodules and no lymph sinuses.

The thymus normally begins to degenerate in the adult animal, although there is considerable individual variation in the extent to which such involution has proceeded at particular ages. The lymphoid tissue becomes progressively less prominent, and connective tissue containing fat cells occupies much of the structure.

Despite many endeavors to discover the exact function of the thymus, there are as yet no clear-cut results. Many students are inclined to regard it as an endocrine gland, but at present there is no good evidence in proof of this conclusion.

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See Appendix for general text references.

CHAPTER IX.

THE INTEGUMENT.

THE covering of the vertebrate body, the integument, is composed of the skin and the various structures, such as scales, claws, feathers, nails, hair, and glands. The integument is in direct contact with the environment and its modifications give protection from injury; prevent loss of body fluids; receive such stimuli as those of touch, pain, or temperature, leading to adjustment to external conditions; aid in the elimination of certain wastes; and resist the entrance of injurious substances.

The skin of all vertebrates is divisible into two layers, an outer layer, the epidermis, composed of stratified epithelium, and an underlying layer of connective tissue, the dermis or corium.

Epidermis.—The epidermis is derived from embryonic ectoderm and is divisible into two regions. The layer of cells which rests on the corium is called the stratum germinativum, or Malpighian layer. Cells of this layer are in contact with the nutritive fluids carried in the corium, and by growth and division they give rise to new cells that are gradually pushed outward to form the superficial second layer, the stratum corneum. As the outermost cells of the stratum corneum are worn away, other cells are added from the stratum germinativum. Invaginations of the stratum germinativum give rise to certain glands which are associated with the skin. Both the stratum germinativum and the stratum corneum are involved in the formation of such structural modifications of the integument as hair, claws, feathers, and nails. The composition of the epidermis varies in different animals and in different regions of the same individual.

Dermis (Corium).—The dermis, or corium, is derived from mesoderm and is composed mainly of fibrous connective tissue, with some intermingling elastic fibers supporting blood vessels and lymphatics. Smooth muscle cells may also be present in the region of connective tissue. Chromatophores, too, are generally distributed in this region. Sense organs and nerve fibers are scattered throughout the corium, and free nerve endings extend into the basal layers of the epidermis.

The corium is continuous with a region of looser fibroelastic connective tissue, the subcutis, which in turn continues with the deeper connective tissue sheaths surrounding the voluntary muscles of the skeleton. Adipose tissue appears in relatively large amounts in the subcutaneous connective tissues of most vertebrates. There are marked variations in the details of skin structure in the different classes of animals.

INTEGUMENT OF FISHES.

The epidermis of a fish is usually composed of stratified squamous epithelium. Low columnar or cuboidal cells occur in the stratum germinativum, polyhedral cells compose the intermediate layers, and squamous cells form the superficial layers. Unicellular and multicellular glands secrete mucus, which forms a protective film over the surface. Scattered in the lower portion of the epidermis are groups of epithelial cells forming simple sensory organs which receive free nerve endings.

The dermis is relatively thin and is composed of fibrous connective tissue lying parallel to the skin surface. Pigment cells are
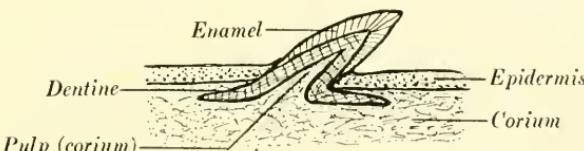
Fig. 81.—Diagram of structure of a placoid scale of an elasmobranch. The enamel develops from the epidermis; the dentine from the corium.

abundant in this region. In some fishes the integument is composed simply of the skin and its glands, but in the majority there are additional structures, the scales, which are formed mainly from outgrowths of the corium.

Scales.— In the Elasmobranchs, multiplication of dermal cells occurs at regular intervals to form a small papilla which projects above the corium and carries along the stratum germinativum of the epidermis. The peripheral cells of these papillæ with the adjacent dermal cells become osteoblasts and secrete a bony plate covering each papilla. The overlying epidermal cells become active and deposit a layer of enamel over this bony dentine base to form the placoid type of scale. With continuous growth, spine-like papillæ project from a flat base in the corium above the general epidermal covering. The central portion of these scales is filled with connective-tissue pulp, which carries blood vessels and nerves. (Fig. 81.)

Such scales are fundamentally the same in origin as the teeth of these fish, and are similar in origin to the teeth in higher verte- brates. Three other general types of scales occur among fishes, namely, ganoid, cycloid, and ctenoid scales. These are products formed in pockets of the corium alone, without marked modification of epidermal covering.

In the skin of the bass many overlapping ctenoid scales project above the general level of the epidermis. The epidermis which is carried out with these plate-like projections is composed of stratified squamous epithelium with a basal layer of cuboidal cells and several intermediate layers of progressively more flattened cells until the superficial layer of squamous cells is reached. (Fig. 82.) The

—*Epidermis*

—*Corium*
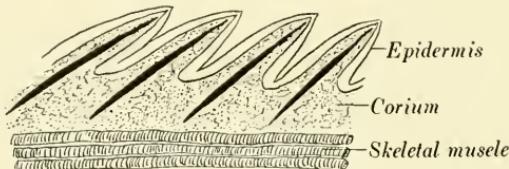
—*Skeletal musele*

Fig. 82. — Diagram of integument of the bass, showing epidermis extending over and between the overlapping scales. The scales are embedded in dense fibrous connective tissue of the corium and above the skeletal muscle shown in longitudinal section.

epidermis rests upon a thin membrane of loose fibroelastic con- nective tissue which separates it from the scales and the underlying very dense fibrous portion of the corium. The epidermis takes no part in the scale formation and is thinner where it folds under the projecting scale. The scale is basally embedded in the dense fibrous connective-tissue region of the corium, where its outline is serrate. Above this region it is surrounded by the loose connective tissue separating it from the epidermis. Chromatophores occur in this loose connective tissue closely surrounding the scales. The scales are formed by the activity of osteoblasts derived from the corium.

INTEGUMENT OF AMPHIBIA.

The skin of amphibians is usually soft and moist and carries no scales. The epidermis is characterized by a stratified epithelium through which numerous multicellular alveolar mucous glands open. (Fig. 83.) The individual surface cells are not desquamated, but there is a periodic shedding of large patches or sheets of cells of the corneum. The corium is thin and may be separated by lymph spaces

from the underlying tissues. The rich supply of blood vessels which extend into the corium facilitate respiratory exchanges through the skin, which in these forms may be the main respiratory organ. The gills of such forms as Necturus are tuft-like projections of skin richly supplied by the capillaries of the corium. In such forms as toads, which are primarily land-living forms, the epidermis is hardened and thickened to prevent loss of moisture.

The skin of the frog is surfaced with stratified squamous epithelim, five to six rows of cells deep. The basal cells are low columnar
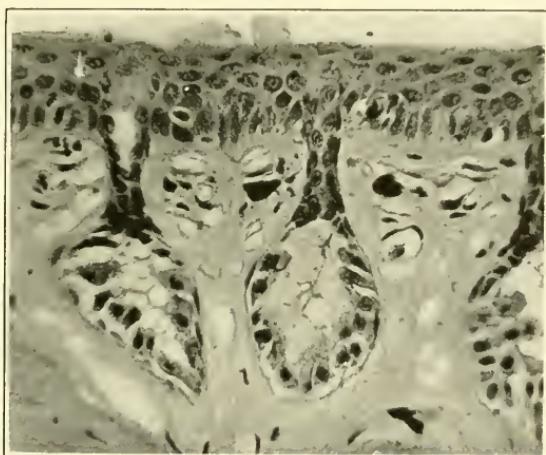
FIG. 83.—Photograph of integument of the frog with an epidermis composed of stratified squamous epithelium and associated simple alveolar mucous glands. Immediately below the epidermis there is a narrow region of loose vascular connective tissue in which contracted chromatophores occur; below the glands the corium is composed of a dense fibrous tissue.

or cuboidal, the intermediate cells flatten out toward the surface, and the superficial scaly cells adhere to one another so as to form a cuticular covering which sloughs off in sheet-like patches. Just underneath the epithelium there is a loose vascular connective tissue in which chromatophore cells are scattered, while beneath this is a zone of more densely organized connective tissue. The deepest region of the corium is densely organized, with parallel collagenous bundles. Alveolar glands extend into the superficial corium beneath the chromatophore network, each gland opening among the basal cells of the epithelial covering. The excretory duct leading from the mouth of the gland forms a channel passing through the epithelium to the surface.

Two types of alveolar glands are differentiated. A smaller mucous secreting gland occurs generally in the connective tissue below the epithelium. Another and larger type, called the poison gland, extends more deeply into the corium and is surrounded by smooth muscle cells. It is composed of cells filled with acidophilic granules; the major portion of these cells disintegrate, liberating the secretion into the lumen and leaving the nuclei scattered along connective-tissue sheath. The function of these glands is protective, their secretions are poisonous and presumably discourage many animals from eating frogs. To the mucous glands are attributed the function of keeping the skin moist.

The skin of Necturus has a stratified cuboidal epithelium forming the epidermis and a network of chromatophores in the connective tissue just below. Among the epithelial cells are scattered spherical cells filled with acidophilic secretion. As in the frog, there are two types of glands. One is a smaller mucous gland, while the other is a larger serous type, in which the contents of the cells disintegrate and fill the lumen with secretion, leaving the nuclei scattered around the periphery.

INTEGUMENT OF REPTILES.

With reptiles the land habitat is assumed, and a dry skin makes its appearance. The epidermis of the reptile is relatively thin and the superficial region in contact with the air is composed of dead, cornified squamous cells. Folds of the cornified epidermis alone form the scales of reptiles. Some reptiles shed their skin periodically, the horny superficial epidermis (the corneum layer) separates from the softer subjacent cell layers so that this dead upper covering of the epidermis is shed in one piece. Glands are restricted to regions about the mouth and anus.

The corium in reptiles is relatively thick and is composed of dense connective tissue, with numerous chromatophores located immediately below the epidermal covering. When bony plates are formed they are derived from the corium in a manner similar to that described in intramembranous bone formation. In most turtles, both scales and bony plates are formed. In turtles and alligators, the tissue of each plate is not continuous with the bony tissue of adjacent plates, so that growth in size of the animal is accompanied by continuous peripheral additions to the individual plates. When claws are present, they are formed by accumulations of the corneal layer of the epidermis.

INTEGUMENT OF BIRDS.

In birds the skin is thin but is covered by scales and feathers formed by the epidermis. The epidermis has a superficial layer of dead cornified squamous cells resting on a few layers of polyhedral cells. The scales covering the legs and the claws of the toes are formed by accumulation of cells in the corneum. Glands are limited to the base of the tail, where the uropygial glands secrete an oily substance used mainly in dressing the feathers. The dermis is composed of dense fibrous connective tissue adjoining the epidermis, but in the deeper region there is a looser fibroelastic connective tissue carrying blood vessels and containing numerous fat cells. Pigment cells are generally absent from the corium, the coloring of birds being due to pigment secreted in the epidermal cells of the feathers. The bill, or beak, of birds is formed by the accumulation of horny epidermal cells.

Feathers. — The beginning of feather development is very similar to that of scale development: a dermal papilla thrusts out with a covering of epidermis and then the base sinks into the dermis. The dermal papilla carries blood vessels and nerves to this epidermal covering which alone is responsible for the feather formation.

INTEGUMENT OF MAMMALS.

Among the mammals, both layers of the skin are usually much thicker than in the forms previously described. Multicellular glands and epidermal formations, the hairs, are abundant. The structure of the epidermis varies considerably in different parts of the mammalian body. In exposed regions where there is considerable friction, the epidermis is extremely thick because of an accumulation of cornified cells in the corneum. Over the body generally the skin proper is broken by numerous projecting hairs, as well as by the ducts of sweat and sebaceous glands derived from the activity of the stratum germinativum. Pigment may be found in the epidermal cells of most mammals.

The dermis composed of interlacing bundles of fibroelastic connective tissue divisible into two blending layers, an upper narrow one, called the subepithelial or papillary layer, and a deeper, denser, wide reticular layer. Immediately below the basal epithelial cells of the epidermis lies a fine network of reticular fibers and reticular cells. These fibers intermingle with the elastic and collagenous fibers to form a network generally paralleling the skin surface.

The dermis projects into the epidermis in the form of ridges and papillæ. In the connective tissue near the epidermis are cells containing pigment granules and resembling the dermal chromatophores which are so very prominent in the dermis of amphibians. Some of the dermal papillæ contain capillary networks furnishing nutriment to this region and to the overlying epidermis. In papillæ adjacent to those possessing capillary networks are a number of types of sensory end-organs.
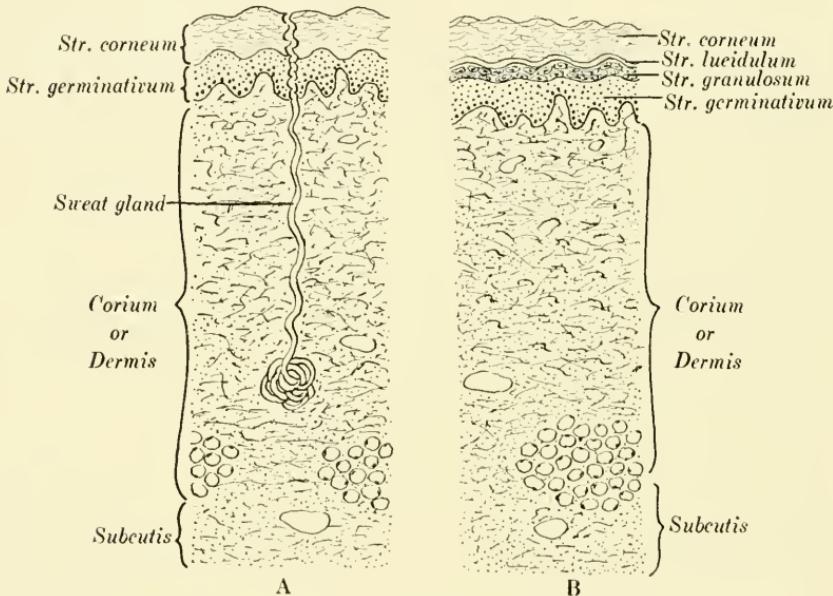
Fig. 84.—Diagrams of skin structure. *A*, from back of hand; *B*, from palm of hand.

Skin of Palm or Sole.—The skin covering the palm of the hand or sole of the foot offers an example of the extreme development of the skin of mammals. (Fig. 84.) The epidermis of these regions presents four distinct strata. Proceeding upward from the dermis these are: the stratum germinativum, the stratum granulosum, the stratum lucidum, and the stratum corneum, or superficial layer. It is to be understood that cells pass through all these layers before being cast off from the surface of the skin, the different layers merely representing various stages in the transformation of the epidermal cell, from the time it leaves the stratum germinativum, until its death and desquamation.

Stratum Germinativum.—The lower border of the stratum germinativum is not a smooth surface because of the projection of dermal

papillæ. Since the outer surface of the epidermis is smooth, its diameter must be thicker at some places than at others. The stratum germinativum, or Malpighian layer, consists of a base of short columnar cells overlying which are several strata of polyhedral cells, the outermost being somewhat flatter than the inner. Frequent cell divisions occur in the cells of the Malpighian layer, and at each division one of the daughter cells is pushed out toward the surface. Since this process is continuous, older cells are constantly being pushed further and further away from the basal layer. Consequently, each cell passes through the three outer zones and is eventually cast off from the surface. So-called protoplasmic bridges connect adjacent cells of the stratum germinativum and have led some to call them "prickle" cells. Skin color depends on different colored
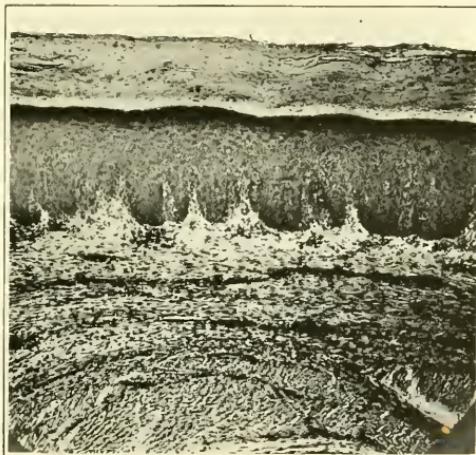
FIG. 85.—Section through the foot-pad of a squirrel, showing four regions of epidermis.

pigment granules in the lower cells of the stratum germinativum. These pigments range through black to yellow and red and may be present only in the lower layer of cells, or may extend to all the cells of the stratum germinativum.

Stratum Granulosum.—This is a zone of several layers of cells outside the stratum germinativum. The cells have a flattened polyhedral shape. Irregular-sized granules which are composed of a substance called keratohyaline represent some definite chemical change in the protoplasm of these cells. The nuclei of the outer cells of this granular zone disintegrate.

Stratum Lucidum.—This layer appears as a bright, clear band just above the stratum granulosum and consists of closely packed

translucent cells. The granules so conspicuous in the granulosum are, in this layer, dissolved into a semifluid colloidal substance called eleidin. (Fig. 85.)

Stratum Corneum.—This is the superficial layer of the epidermis composed of dead scale-like cells in which the eleidin has become keratin and the nuclear regions appear as clear spaces. These cells are constantly being shed or desquamated.

In other regions of the skin, however, these four zones do not normally appear and only the stratum germinativum and the stratum corneum are distinct.

Sweat Glands.—Distributed widely through the skin of some mammals are the simple coiled tubular sweat glands. (Fig. 84.) Their coiled portion lies well down in the reticular layer of the dermis, or even in the subcutaneous region. The excretory duct formed by cuboidal cells runs a more or less straight course. In the epidermis the duct is merely a spiral passageway between the cells. The internal twisted secreting portion is composed of pyramidal cells, each with a basally placed nucleus and granular or vacuolated cytoplasm. The free end of the cells adjacent to the lumen may swell with secretion and become detached from the basal nucleated part to fill the lumen. The secretion is watery and may be slightly colored from pigment present in the cells. In cats, rats, and mice the sweat glands are restricted to the toe pads; in rabbits, to the region around the lips; in deer, to a region near the base of the tail; in the hippopotamus, to the inside of the ears; and in the cow, to the surface of the snout.

These glands play a part in the regulation of body temperature and the excretion of wastes.

Hairs.—Hair development is similar to that of feathers and scales. A hair originates from groups of cells in the deep layers of the epidermis which grow down into the subjacent dermal connective tissue and produce an epithelial rod at an oblique angle with the surface. From these epithelial cells surrounded by the connective tissue of the dermis, shafts of hairs are formed and grow out beyond the surface of the skin. The epithelial cells and surrounding connective tissue of the skin form the hair root. (Fig. 86.)

The root is surrounded by the hair follicle, a blind tube-like sac, internally composed of epithelial sheaths, and surrounded by connective-tissue sheaths. Indenting the base of the follicle there is a knob-like extension of vascular connective tissue called the hair papilla. Cells multiplying in the basal portion of the epithelium

above the papilla form the shaft, which, with its differentiating and cornifying cells, is thrust upward into the canal leading through the epidermis.

Between the hair itself and the follicle wall is a space, and into this, not far from the surface, opens the excretory duct of a sebaceous
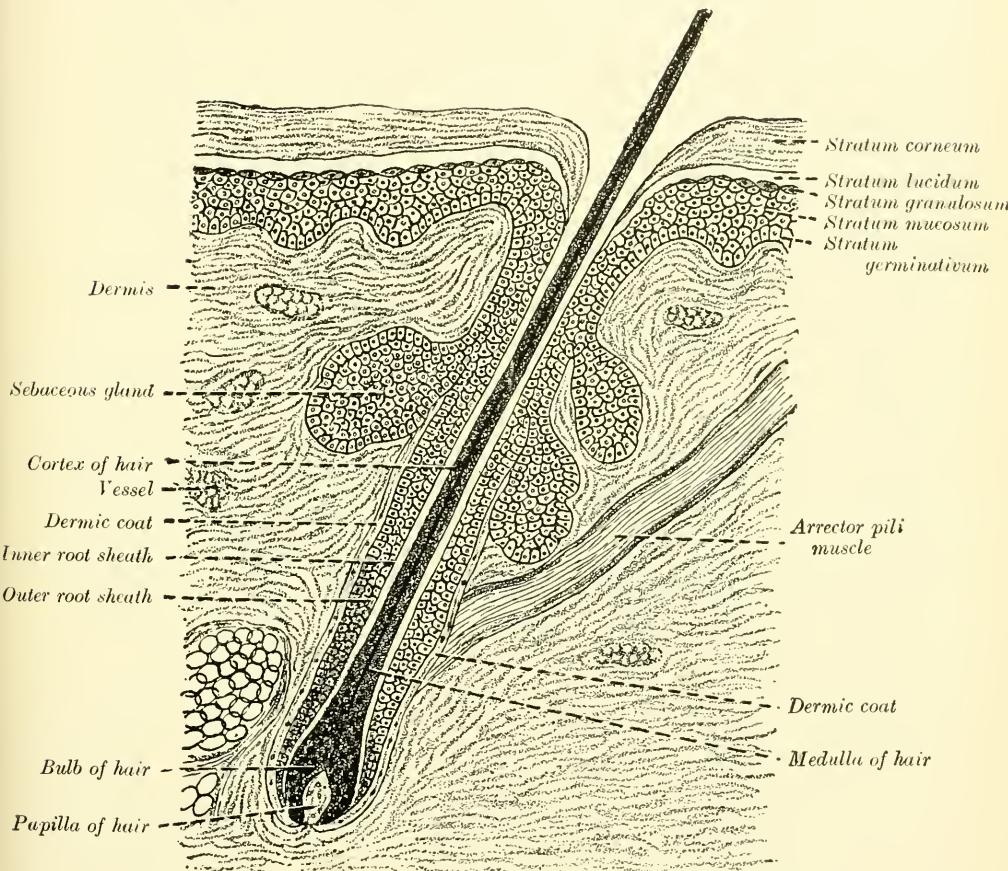
FIG. 86.—Diagram of skin structure in region of a hair.   (Gray's Anatomy.)

gland.   The epidermis of the follicle, from the surface as far down as the sebaceous gland, has the usual epidermal layers of the skin; but further down, first the corneum, and then the granular layer, disappears until only the Malpighian layer, or stratum germinativum, persists in the deeper portion of the follicle.   This persisting layer thins down to form the external root sheath around

the shaft of the hair; further toward the base of the follicle, a special epithelial layer, the inner root sheath, surrounds the root, and separates it from the external root sheath. At the enlarged base of the follicle the sheaths blend with the root into a wall of indifferent epithelial cells above and around the papilla, and from these cells the hair continues to develop. It is evident that cross-sections made through a hair follicle at different levels will show distinctly different compositions.

Hairs are set obliquely in the skin, and on the acute angle side there is a small strand of smooth muscle, the arrector pili muscle, which extends through the dermis from the side of the follicle diagonally upward toward the epidermis. When this muscle contracts the hair is pulled more erect to form a temporary ridge in the skin adjacent to the hair. When such contractions occur generally over the body they produce the condition of the skin known as "goose flesh." In man there is a constant falling-out of hair and growth of new hairs, quite independent of the seasons; in most mammals the shedding of hair takes place annually, in late spring or early summer.

Sebaceous Glands.—Between the arrector pili muscle and the hair follicle there is usually a sebaceous gland which is simple branched, alveolar in type. The gland is composed of large, clear cells derived from the epithelia of the outer root sheath, and is surrounded by a capsule of connective tissue derived from the follicle sheath. The excretory duct is relatively wide and has a layer of stratified squamous epithelium. (Fig. 86.)

The alveoli are filled with stratified cells which are relatively small and mitotically active basally. Toward the center of the alveolus, one can follow the accumulation of fat globules in the cytoplasm with concomitant increase in the size of the cell. Ultimately the entire cell disintegrates and the secretion, together with the disintegrating products of the cell itself, is liberated at the side of the hair near the skin surface as an oily compound, the sebum. After such disintegration of the cells and the discharge of the sebum, regeneration of new epithelium of the gland takes place from the basal cells. Possibly the muscular contraction of the arrector pili muscle aids in the discharge of the secretion at the neck of the hair follicle.

Meibomian glands are modified sebaceous glands which occur in mammals along the edge of the eyelids. They produce a

secretion liberated at the base of the eyelash. Somewhat similar *ceruminous* glands lining the external auditory meatus secrete a waxy substance. Most mammals have mucus-producing and lubricating epidermal glands associated with the skin of the external reproductive structures. In the skin near the anus of some forms there are modified sebaceous glands producing odoriferous secretions.

Mammary Glands.—These glands, which are for the most part located in the dermis, arise from invaginations of the embryonic skin. They form in both male and female, but normally develop to functional maturity only in the female and undergo regressive

FIG. 87.—Mammary gland of human in intermediate stage of activity, showing two lobules with an interlobular duct breaking into two intralobular ducts that extend into the secreting end-pieces. A dense connective tissue surrounds the lobules and groups them into lobes.

changes in the male. Each mammary gland (Fig. 87) is compound alveolar in form. The excretory ducts open in the nipple, which is a conical extension of connective tissue covered with epidermis. The alveolar portions of the mammary glands are large and composed of pyramidal cells, but there is considerable variation in size depending on the secretory state of the gland. When active, fat globules accumulate in the cytoplasm toward the free end of the cells and the enlarged terminal portions are discharged into the lumen. The remaining nucleated basal portion of each cell soon repeats the process. Secretions accumulating in the lumen pass along small ducts which open into the larger lactiferous ducts. The number

10

of these ducts in each nipple varies from one to many. They open to the surface at the end of the nipple, where the stratified squamous epithelium of the covering skin continues inward to line their outermost portion. Before reaching the nipple, the lactiferous ducts dilate into reservoirs for the storage of the secreted milk. In the inactive state the alveoli are collapsed and the gland is composed mainly of connective tissue.

Nails.—The nails are comparatively simple derivatives of the skin, which cover the dorsal surface of the tips of the fingers and toes of primates. (Fig. 88.) They are horny plates, slightly convex and
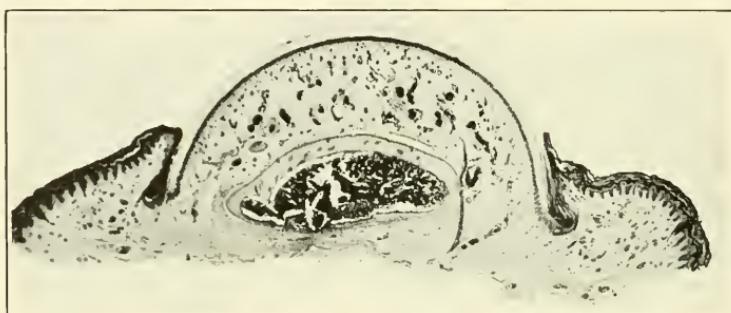
Fig. 88.—Cross-section of the finger tip and nail of human, showing nail bed, grooves, nail, and skin of the finger.

roughly rectangular. The skin underneath the nail bed rounds laterally and proximally into folds which form the nail wall. The overlapping of these walls on the nails make deep proximal and shallow lateral nail grooves in which the nail lies. Under the proximal wall the nail is soft and is composed of stratified squamous epithelium corresponding to the stratum germinativum, proliferation from which gives rise to cells that harden as they push outward. The nail plate is formed of fused, flat, scale-like, cornified, epithelium cells, and corresponds to the stratum corneum of the skin. In the proximal region the underlying epithelium is thicker and forms the matrix from which new nail formation takes place.

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See Appendix for general text references.

CHAPTER X.

THE RESPIRATORY SYSTEM.

In describing the blood and integument we have already considered one of the means whereby respiratory needs are supplied. Depending upon the environment of the animal in question, there are certain other arrangements of tissues to accommodate gaseous exchanges required by metabolism.

THE RESPIRATORY SYSTEM OF FISHES.

In the case of fish, respiration is effected mainly by means of gills, which are specialized structures arising from the pharyngeal region to accommodate respiration in water. Water enters through the mouth, passes into chambers surrounding the gills, and bathes them before passing outward through external gill slits. A typical gill is composed of a median septum supporting two lamellæ of connective tissue and muscle and an exposed epithelial surface. The septa are supported by cartilaginous or bony arches. The lamellar surface is usually much folded and near it is an extensive capillary supply bringing the blood close to the water for the exchange of gases through the epithelial membranes.

The air sac or swim bladder appearing in the majority of the higher fishes is formed as a thin-walled pouch from the esophageal region and is lined with thin epithelial membranes. It has been considered by some as being associated with respiration and also as a hydrostatic organ.

THE RESPIRATORY SYSTEM OF AMPHIBIANS.

In amphibian larvæ and in adults of all mature water-dwelling forms, thin membranous external gills are developed as epidermal folds. (Fig. 89.) They are lost in the adults of those species adapted to terrestrial life. These membranes are well supplied with blood vessels (Fig. 90) and serve for respiration during aquatic life. The skin is also vascularly equipped and takes part in respiration in many forms.

In terrestrial amphibians, air is taken in through the nostrils with the aid of muscles of the mouth and throat, and passes into the two internal sac-like diverticula of the pharynx, the lungs. (Fig. 91.)
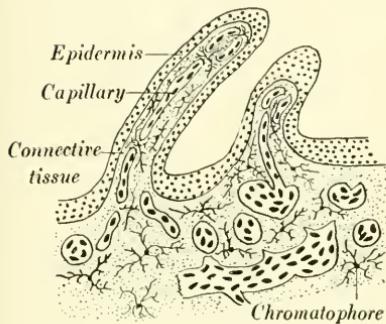
FIG. 89.—Diagram of the gill filament of Necturus.
FIG. 90.—Diagram of Necturus gill filament more highly magnified.
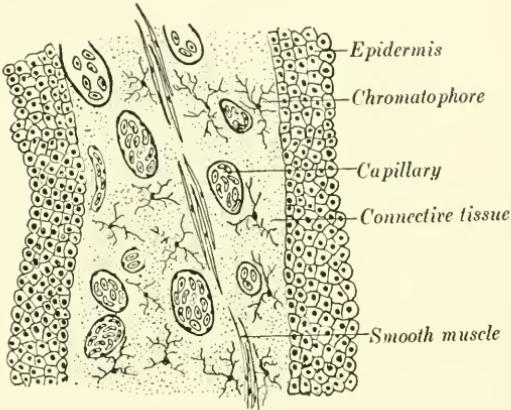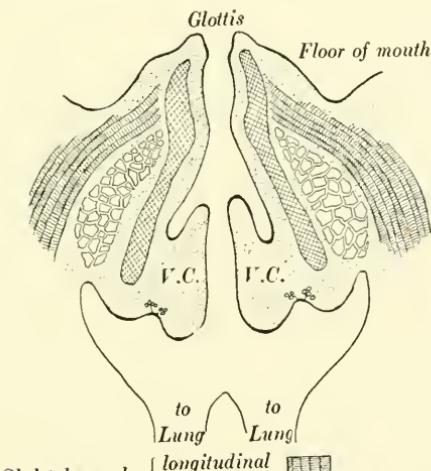
FIG. 91.—Glottis, vocal cords and entrance to lungs of frog.

In the water-dwelling forms the lungs are like the air sacs of the fishes in their simplicity. (Fig. 92.) In the frog and other terrestrial forms the internal surface is lined by simple squamous or cuboidal

epithelium, and the lung develops simple alveolar cavities which
increase the respiratory area.
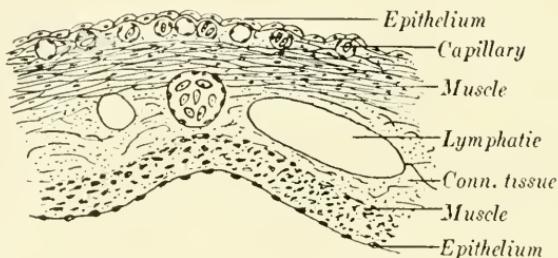
Fig. 92.—Wall of the lung of Necturus.

THE RESPIRATORY SYSTEM OF REPTILES.

With the reptiles the lungs take on the entire burden of respiration
and dispense with the accessory skin system of amphibians.   Other
structures associated with the mammalian lung also develop, the
nasal passage connecting with the pharynx and cartilaginous rings
about the trachea.   The lungs themselves become increased in
respiratory capacity by horizontal and vertical folds bearing the
alveolæ with an accompanying increase in the vascular supply.
(Fig. 93.)   The stratified ciliated columnar epithelial lining of the
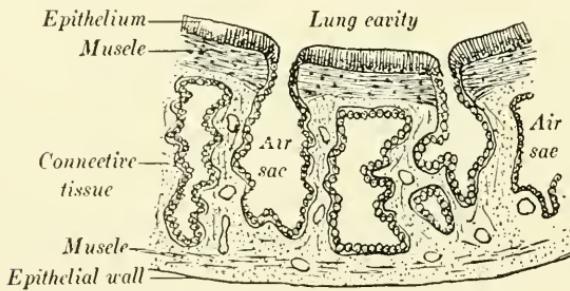
Fig. 93.—Wall of lung of water snake (Natrix).

pharynx becomes a simple epithelium lining the respiratory surfaces
of the lungs.

THE RESPIRATORY SYSTEM OF MAMMALS.

When the mouth of a mammal is closed, air is taken in through
the nasal passages to the pharynx, thence into the larynx and on to
the trachea.   The trachea divides into two bronchi, one to each
lung.   From the bronchi, air passes through smaller branches to

bronchioles and eventually reaches the microscopic end-pieces, the respiratory alveoli, where gaseous exchange takes place.

The nose is an organ composed of bone, cartilage, muscle, skin, and epithelial membranes, so constructed as to provide open ways for the passage of air. The olfactory area of the passage has epithelium composed of three types of cells. There are small basal cells and long, thin supporting cells; scattered between these cells are long fusiform olfactory cells.

The nasal cavity continues into many irregular spaces, or sinuses, lined with a thin epithelial membrane. The nasal passage continues posteriorly into the pharynx, where the epithelium consists of pseudostratified ciliated epithelium. Many of the long superficial cells are goblet cells, and the surface of the membrane is generally well moistened with mucus. Under the epithelium is fibroelastic connective tissue carrying lymphatics, blood vessels, nerves, and small mixed mucoserous glands.

The Larynx.—The larynx is posterior to the pharynx in quadruped mammals and is sometimes called the voice box, for it contains the vocal cords. It has a skeleton of a number of cartilaginous pieces, some of them single units, others occurring in pairs. In general, these cartilages are of the hyaline variety. They are covered with perichondrium which merges with the fibroelastic connective tissue forming the external covering of the larynx. Many small muscles of the voluntary striated type are present. The cartilages are covered with loose fibroelastic connective tissue which may be called a submucosa or tunica propria. It contains much lymphoid tissue and possibly scattered single lymph nodules. The epithelium of the mucosa is of the pseudostratified ciliated variety, except over the vocal cords and under the epiglottis where it is stratified squamous.

The vocal cords are a pair of shelf-like tissue masses extending from the lateral walls of the larynx into the laryngeal cavity. Each vocal cord has an external skeletal muscle mass. Internal to this, toward the lumen, is elastic tissue and the stratified squamous epithelium covering the vocal cords.

The Trachea.—The trachea is a dilated tube extending from the larynx to the bronchi. It is just dorsal to the skin of the neck in the mid-ventral line and dorsal to it is the esophagus. Laterally the muscles and blood vessels of the neck are associated with fibroelastic connective tissue which also forms the outer covering of the trachea. The thyroid gland lies against its ventral surface

just back of the larynx.   As it enters the thorax, the trachea
divides into two branches, the bronchi, one of which enters each
lung.

Embedded in the tracheal connective tissue are the rings of
cartilage which keep this air passage dilated.   In old animals they
may undergo partial ossification.   The cartilages are not complete
rings, but usually have an open space between the ends adjacent
to the esophagus.   This space between the ends of the cartilage is
filled by a band of smooth muscle and connective tissue.   The
cartilages are enclosed in perichondrium and the superficial portions
of the perichondrium merge with
the adventitial connective tissues
and with similar fibroelastic con-
nective   tissue   lying   between
adjacent cartilages.   The internal
connective tissue may be called a
submucosa and is rich in elastic
fibers.   In   it   are   many   small
compound   mucous   and   mixed
mucoserous   glands.   The   sub-
mucosa also contains many scat-
tered   lymphocytes   which   may
be organized into lymph nodules.

The mucosa is composed of
ciliated   pseudostratified   epithe-
lium,   in   which   are   numerous
goblet   cells   which   add   mucus

Epithelium

Connective tissue

Gland

Submucosa

Perichondrium

Cartilage

Perichondrium

Adventitia
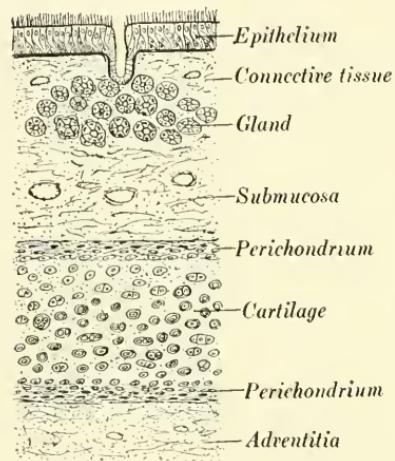
FIG. 94.—Mammalian trachea cross-
section.

to that from the submucosal glands, thus keeping the internal
surface of the trachea moistened.   (Fig. 94.)

The Lungs.—Since the respiratory system of mammals arose
embryologically as an invagination from the embryonic foregut, the
trachea represents the principal excretory duct which divides pos-
teriorly into two bronchi which branch into bronchioles.   The lungs
are invested with a thin double membrane, the pleura, similar in
structure to the peritoneum, with a potential space between the two
membranes.   The pleural membrane adjacent to the lung contains
much smooth muscle in its connective tissue and dips down into
the spaces between adjacent lung lobes to connect with the con-
nective tissue between the lobules.

Bronchi and Branches.—Each bronchus has about the same
structure as the trachea but the size of the tubes decrease as

the branching increases. (Fig. 95.) Histological changes also
appear. The cartilage rings change to flat cartilage plates which
become smaller and smaller in the branchings until finally no
cartilage supports the smallest bronchioles. Patches of smooth
muscle arranged concentrically around the tube appear just within
the cartilage plates in the tunica propria. As the branches decrease
in size the muscle increases proportionately until in the very
small or terminal bronchioles there is no cartilage but a distinct
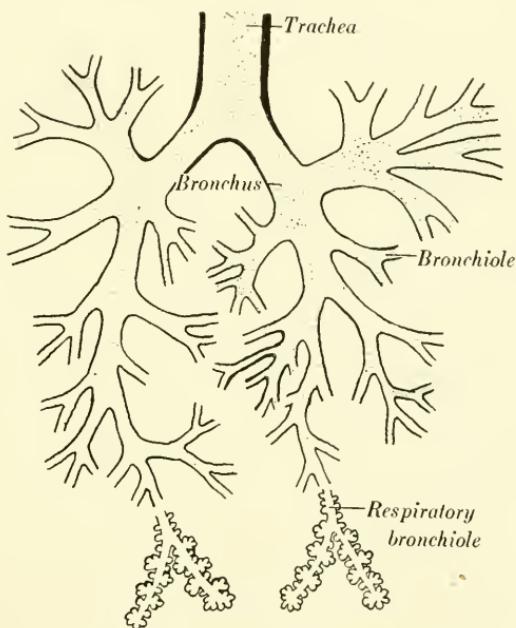
Fig. 95.—Bronchial tree of mammal.

circular sheet of smooth muscle is present outside the tunica propria.
The epithelium changes gradually from a pseudostratified variety
until in the smallest bronchioles there is but one layer of columnar
ciliated epithelium. The goblet cells have disappeared also. The
glands in the tunica propria, which in the larger bronchi were so
numerous, have decreased so that there are none in the smallest
branches. The mucosa and tunica propria in smaller branches
show longitudinal folds.

Terminal bronchioles (Fig. 96) are separated from the respiratory
tissue by their adventitia, a thin layer of fibroelastic connective
tissue. The tunica propria of connective tissue is rich in elastic

fibers arranged lengthwise of the tube and supports the mucosa of ciliated columnar epithelium lining the lumen.

The lung is composed of many lobules which are somewhat conical or pyramidal in shape and are composed of a number of terminal bronchioles and their branches. Adjacent lobules are separated from each other by interlobular connective tissue.

Each terminal bronchiole divides into two or more *respiratory bronchioles.* (Fig. 97.) Near its origin, the mucosa of this subdivision has ciliated columnar epithelium; distally the cilia are lacking and
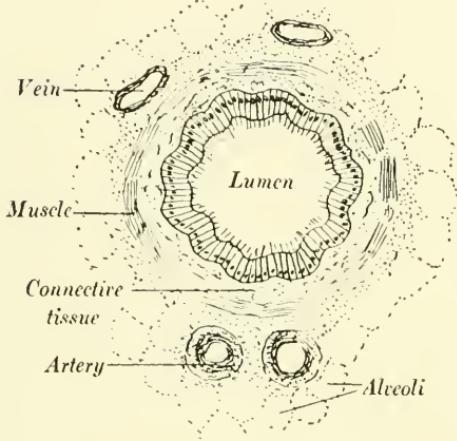
Fig. 96

Fig. 97

Fig. 96.—Diagram of section of a terminal bronchiole, the lumen lined with ciliated columnar epithelium.

Fig. 97.—Terminal bronchiole of mammal and its branches.
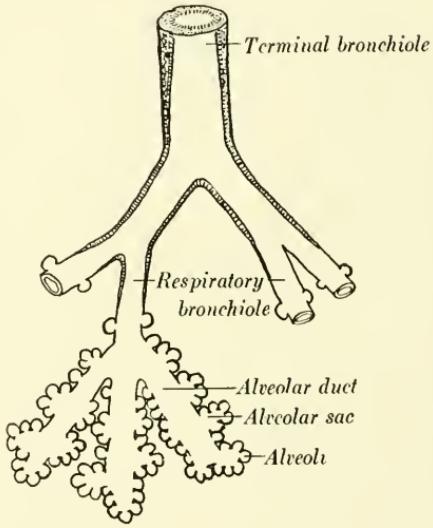
the cells are cuboidal. Outside the mucosa the wall is very thin, consisting of fibrous tissue containing numerous elastic fibers and smooth muscle cells. It has a few small saccular diverticula, or pulmonary air sacs, whose wall apparently consists of flat, squamous epithelium invested with a rich capillary network where gaseous exchanges can take place.

At its distal end the respiratory bronchiole divides into two or more short, thin-walled, irregular *alveolar ducts.* The simple squamous epithelium of the thin wall is supported by a very small amount of fibrous tissue with a few scattered elastic fibers and smooth muscle cells. The wall is broken along its length by many

saccular diverticula, either alveolar sacs or single pulmonary alveoli. (Fig. 98.) The fibrous tissue, elastic fibers, and smooth muscle cells are chiefly in the wall of the alveolar duct, around the openings to the *alveolar sacs* and *pulmonary alveoli.* The alveolar sacs are not single bladder-like enlargements, but each consists of a cluster of respiratory or pulmonary alveoli which are homologous with the secretory end-pieces of a gland like the parotid. Alveolar sacs and respiratory alveoli are mere sacs whose walls appear to consist of squamous epithelium and re-
ticular tissue. The alveoli
often contain large cells called
dust cells, since they contain
black particles. Their source
is debatable.

Each pulmonary unit, there-
fore, consists of a respiratory
bronchiole and its alveolar sacs
and pulmonary alveoli. The
air sacs and alveoli are in-
vested with a fine, close-
meshed network of large capil-
laries, so thick-set that the
spaces between adjacent capil-
laries is less than the width
of the capillaries. Between
adjacent alveoli there is also
a rich meshwork of reticular
fibers, some elastic fibers, and
scattered smooth muscle cells.

Fig. 98.—Photograph of the lung of a rabbit, showing a small bronchiole in upper right. In the center a respiratory bronchiole is shown breaking into two alveolar ducts and their alveoli.

Vascular Supply.—The lungs are supplied by two sets of blood vessels, namely, (a) the bronchial arteries and veins, and (b) pulmonary arteries and veins. The bronchial vessels arise from the systemic system and their branches extend in the connective tissue down into the interlobular connective tissues. The pulmonary artery arises from the right ventricle and has a branch to each lung, where its branches follow those of the bronchi. Alongside a terminal bronchiole is a very small branch of a pulmonary artery. This branches as the respiratory bronchioles are formed, so that near the alveolar ducts and sacs the pulmonary arterioles break into capillaries which invest the air cells. At the lateral borders of the alveoli of a pulmonary unit, the small venules originate which

unite to form small veins of the pulmonary venous system. In a section of a lung, the branches of the pulmonary vein are somewhat distant from a bronchiole. The pulmonary venous system carries to the left auricle blood partially depleted of carbon dioxide and enriched with oxygen to be distributed to all organs and tissues.

The lungs are innervated by branches of the sympathetic and vagus (tenth cranial) nerve. The abundant supply of smooth muscle and elastic tissue present play an important part in the recurring movements of inspiration and expiration. In addition to these are the muscular diaphragm and the intercostal muscles which play a rôle in these movements.

Before birth the lung resembles a compound tubulo-alveolar gland, the terminal pieces being lined with cuboidal epithelium. At birth, the contact of the air with the body usually acts as a stimulus to start up the series of inspirations and expirations that continue until death. With the first few inspirations, the inspired air fills and expands the lungs and the cuboidal cells of the terminal pieces are flattened.

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See Appendix for general text references.

CHAPTER XI.

THE DIGESTIVE SYSTEM.

Arising from the simple embryonic invaginations which form the fore and hind gut, there develops in vertebrates a relatively elaborate system for service in the digestion, absorption, and defecation of food. This system is composed not only of the long canal that extends from mouth to anus, or cloaca, but includes also the tongue, teeth, a few large and a great many small glands derived from embryonic outpocketings of the endodermal (epithelial) lining.

Food is taken in at the mouth, where it is usually subjected to the mechanical process of mastication by the tongue and the teeth. The secretions of the glands of this region pass into the oral cavity to aid in lubricating the food for passage. Food thus crushed and lubricated passes into the esophagus, which conducts it to the stomach, where digestive juices are poured upon it from the glands of the stomach walls. Very little, if any, absorption occurs in the stomach where the food is stored temporarily and is actively worked upon by digestive ferments (enzymes). It is passed from the lower end of the stomach by peristaltic waves into the small intestine, whose surface is greatly increased by folds. Secretions from small glands in the intestinal wall, together with the secretions from the pancreas and the liver, which are introduced here by ducts from these two organs, are all mixed with the food mass in the lumen. Under the combined chemical activities of the secretions added to it, the food is broken into products which are capable of being absorbed through the epithelial wall of the intestine. Passing through the small intestine the unabsorbed portion of the food enters the large intestine where absorption of water takes place. The glands lining the walls of the large intestine are probably mainly active in lubricating unusable materials and waste by-products of digestion for passage and elimination.

THE MUCOUS MEMBRANE.

The intestinal tube extends from the lips to the posterior aperture. There is a line of demarcation at each of these places where the skin ends and the membrane lining the cavity of the intestinal tract

begins. This lining is a mucous membrane, or mucosa. A mucosa
consists of at least two different tissues: an epithelial membrane
and the connective tissue, or tunica propria, upon which it rests.
A third membrane of smooth muscle may also be present and form
a muscularis mucosæ.

A typical mucous membrane, therefore, consists of an epithelium,
a tunica propria, and a muscularis mucosa. Mucous membranes
rest upon a submucosa, which is a loose fibroelastic connective-tissue
layer. The latter is extensible, thus allowing for the passage or pres-
ence of quantities of food in the tract and passively contracts as the
food disappears. The apparent thickness of the submucosa depends
usually on the distention of the canal. Where no muscularis mucosa
is present the connective tissue just underneath the epithelium is
more dense and corresponds to a tunica propria; the deeper zone
which corresponds with the submucosa is more loosely organized.

THE LIPS.

A longitudinal vertical section through the lip, perpendicular to
the surface, shows that the outer surface consists of skin tissue
with an epidermis of stratified epithelium and a dermis of a dense
fibroelastic connective tissue. Hairs, sebaceous glands, and sweat
glands or other typical skin features are present, depending on the
animal.

These structures change at the inner surface of the lip proper,
where the epithelium becomes gradually thicker and loses the
integumental features. Small subepithelial connective tissue papillæ
appear. Where the inner surface is continually moistened by the
fluids present in the mouth, the epithelium is still thicker and the
connective tissue papillæ are more pronounced. The epithelium
of the oral surface rests upon a denser connective tissue (tunica
propria) which emerges into a more loosely organized, deeper con-
nective tissue (submucosa), supporting the larger blood vessels,
lymph vessels, and nerves. Small compound glands of the mucous-
serous type are usually present, those grouped in the submucosa
near the margin of the skin area being called labial glands, and
others further back are known as buccal glands.

The region between the dermis of the skin surface and the sub-
mucosa of the oral mucosa is composed mainly of voluntary muscles.

THE ORAL CAVITY.

This region is lined with stratified epithelium resting upon a subepithelial connective tissue which is thicker in some places than others. In the hard palate, when present, the mucous membrane is in close relation with bone, but over the jaws striated muscle lies below the mucous membrane. Mucous, serous, and mixed mucoserous glands are often present. Reptilian, avian, and mammalian oral epithelium is usually stratified squamous in character. The mucous membrane which lines the oral cavity of the frog is covered with stratified ciliated columnar epithelium in which goblet cells are numerous. There are also frequent pocket-like involutions lined with large goblet cells. In the tunica propria there is diffuse lymphoid tissue and occasionally a lymphoid condensation.

Teeth. — Very conspicuous as oral modifications are the teeth of mammals. They are hard structures derived in part from epithelium and in part from connective tissue covering the jaws. They develop from large papillæ of connective tissue covered by epithelium. Both tissues undergo a peculiar hardening process through chemical transformations and deposits. The development of the teeth resembles that of the placoid scales of Elasmobranchs. The gum consists of muscle, connective tissue, and stratified squamous epithelium.

Each tooth is divisible into a crown which projects above the gum, and a root, or roots in cases of the larger teeth with divided basal insertions, which tapers to fit into an alveolus or pocket in the jaw bone. The tooth is composed mainly of dentin, which surrounds a pulp cavity and is thickest in the crown region where it is covered by an epithelial derivative, the enamel. Below the gum level, a thin layer of cementum covers the dentin of the root. The dentin and cementum resemble bone in structure, but the enamel is formed mainly of deposits of inorganic (only 3 to 5 per cent organic) salts in the epithelial cells and is a harder material than bone. A canal passes through the root carrying blood vessels and nerves to the pulp cavity.

Tongue. — Although the tongue is generally represented in all vertebrates, it varies widely in the extent to which it develops.

Among the fish it is poorly represented, and is called a primary tongue. Such a tongue is composed of a projection of dense connective tissue from the floor of the mouth and is covered by a mucous membrane similar to that of the oral cavity.

The urodeles show evidences of the formation of a secondary extension of the primary tongue, and this consists of muscle, connective tissue, and epithelium, together with glands that secrete a sticky mucous substance used in capturing food. When at rest the tongue of the frog lies along the mid-line of the mouth's floor, being attached in front and free behind. It can be flipped out, and when extended assumes a spoon-like form for catching its moving prey. The upper and lower surfaces are covered by stratified columnar epithelium. The epithelium of the upper surface of the resting tongue is comparatively thin, with many
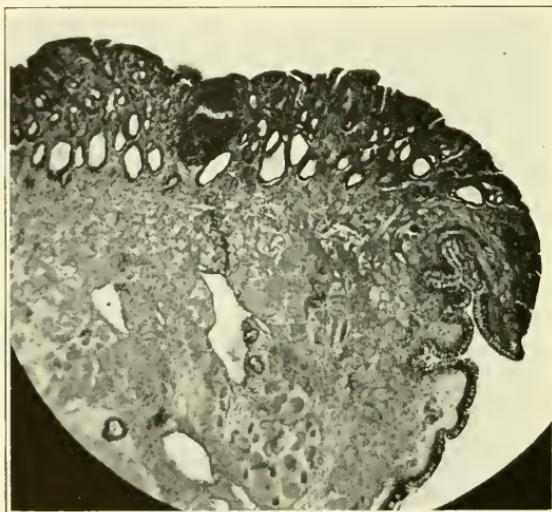
FIG. 99.—Photograph of the tongue of the frog. The upper portion shows the glandular lower surface of the tongue.

goblet cells and may be folded to form numerous papillæ. The lower surface has a great many simple or branched tubular glands with dilated ends separated from each other by a minute amount of connective tissue. (Fig. 99.) The superficial epithelium is of the stratified columnar type, but the glands are lined with simple columnar cells, mainly mucous in nature. Considerable diffuse lymphoid tissue may be found below the epithelial surface, and occasionally a lymphoid condensation resembling a nodule. In the connective tissue below the mucosal covering are bundles of striated muscle fibers. The main blood vessels and nerves follow a central course through the tongue.

Chelonia and Crocodilia have a fleshy secondary tongue capable

of limited movement. In snakes the tongue is a slender bifurcate muscular organ that is usually retractable within a sheath in the base of the mouth. It is covered with stratified squamous epithelium, below which a thin coat of connective tissue with chromatophores adjoins the underlying muscles. The majority of the muscle fibers run longitudinally. The main blood vessels and the large nerve trunks run through the center of the tongue on either side of a median band of perpendicular muscle. It apparently functions as an olfactory receptor in snakes.

The mammalian tongue is an active organ composed, in greater part, of voluntary muscle sheathed by a mucous membrane continuous with that of the mouth and pharynx. Extrinsic muscles enter the tongue posteriorly and connect it with the cartilages of the throat. The body of the tongue is composed of the intrinsic muscle, or tongue muscle proper, which is directly concerned with the movements of the tongue.

A vertical partition, the median or lingual septum, of dense fibroelastic connective tissue extends from the lower to the upper surfaces and from the base to the tip dividing the tongue into two equal lateral portions. Small bundles of muscle fibers of the skeletal type are arranged longitudinally, vertically, and transversely, and cross each other at right angles. The muscle fibers and bundles are separated from each other by thin sheets of fibroelastic and adipose connective tissues in varying amounts.

Between the muscular portion of the tongue and the epithelial sheath there is a narrow region of subepithelial connective tissue from the surface of which projections extend into the overlying epithelium. This connective tissue is continuous with the connective tissue separating the muscles of the tongue proper.

The epithelium of the mucosa of the tongue is of the stratified squamous type throughout. The surface is smooth for the most part along the sides and on the ventral surface and is similar to the lining of the mouth with which it is continuous. The two-thirds toward the tip is roughened by papillæ of varying sizes and shapes (Fig. 100), formed by upward projections of the subepithelial connective tissue that carry with them a covering of stratified squamous epithelium. The anterior region of the dorsal surface of the tongue bearing them is called the papillary portion. The posterior third of the tongue may lack papillæ of any kind, but may have lymphoid tissue forming masses called the lingual tonsils beneath the superficial mucous membrane.

11

In the papillary region four types of papillæ may be found, namely, filiform, fungiform, foliate, and circumvallate.

Filiform papillæ are the most numerous and occur in rows throughout the papillary zone. Each is formed by a conical core of connective tissue that projects well out beyond the general surface of
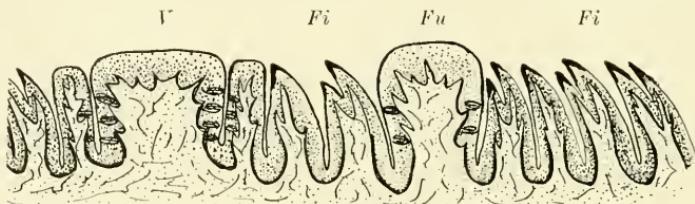
Fig. 100.—Diagram of three different types of tongue papillæ. *V*., vallate; *Fi*., filiform; *Fu*., fungiform.

the tongue and is covered by the stratified squamous epithelium. The superficial cells of the epithelium of these papillæ in some animals become heavily cornified and present a rough, file-like surface that is useful in masticating food.
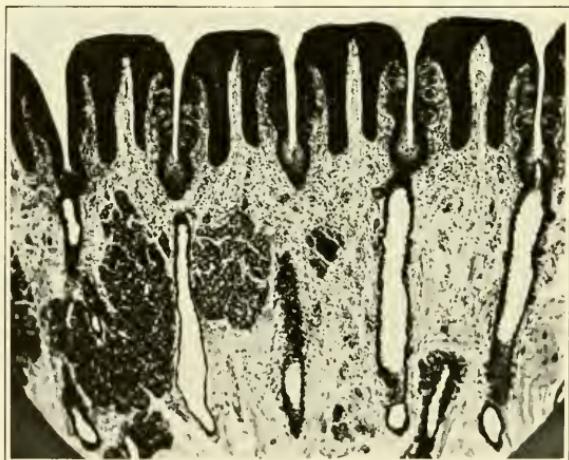
Fig. 101.—Section through foliate papillæ of rabbit, showing von Ebener's glands in the underlying tissues.

Fungiform papillæ are much fewer in number than the filiform and are scattered irregularly over the papillary region among the filiform papillæ. They are formed by extensions of the connective tissue that broaden out as they rise above the general level of the tongue. The epithelial covering is thin, so that these papillæ appear

red, due to the blood of the underlying connective tissue showing
through. Taste-buds may appear in the side walls of the fungiform
papillæ.

Foliate papillæ (Fig. 101), as the name implies, are leaf-like and
occur along each lateral margin of the tongue toward the rear of
the papillary zone. They occur in varying numbers as narrow,
parallel, consecutive, vertical ridges separated by furrows. They
are poorly defined in man but well represented in such animals as
the rabbit and opossum. Here they may be seen as an oblong patch
on each side of the tongue. The primary papilla of connective
tissue has three definite secondary projections over which the
epithelial coat forms a smooth sur-
face. Numerous taste-buds occur
in the lateral epithelial walls of
these papillæ. (Fig. 102.)

The circumvallate or vallate
papillæ occur just in front of the
foramen cecum, which is a shallow
depression posterior to the papil-
lary region. They are arranged in
a V-shaped pattern, with the apex
of the "V" toward the foramen
cecum, are few in number, and are
the largest type of papillæ. Each
has a knob-shaped form with a flat
top and is surrounded by a deep
furrow or fossa, so that the papilla
does not project above the general
level of the tongue. The primary

Fig. 102.—Photograph of taste-
buds in the walls of the foliate papillæ
of the rabbit.

connective-tissue papilla is divided into a number of smaller sec-
ondary papillæ covered with stratified squamous epithelium.
Numerous taste-buds occur in the epithelium of the side walls
and in the epithelium of the opposite wall of the fossa.

The blood vessels and nerves for all types of papillæ are sup-
ported in the connective-tissue core. Fibers of the gustatory nerve
pass to the sensory cells of the taste-buds located in the various
papillæ. Each taste-bud is somewhat cask-shaped; the epithelial
wall is formed by flat fusiform epithelial cells that are arranged like
the staves of a cask. One end of this cask-like bud is open and faces
the fossa (furrow) about the papilla. The space within the wall
of flat epithelial cells is occupied by two kinds of specialized epithe-

lial cells derived from embryonic ectoderm. There are long spindle-shaped cells, called supporting cells, and among these are long, tapering, sensory cells with hair-like external tips that project into the opening to the fossa. Each of the sensory cells is connected basally with a fiber of the gustatory nerve. Substances entering the mouth in solution come into contact with the hair-like tips of the sensory cells, which when stimulated give the sense of taste.

Many serous and mucous glands are present in the tongue. Serous glands, known as von Ebener's glands, are numerous in the neighborhood of the foliate papillæ and among the muscle masses. The ducts from these glands open to the exterior through the bottom of the fossa between the adjacent papillæ. (Fig. 101.) In the region of the circumvallate papillæ there are present in many mammals mucous as well as serous glands.

Salivary Glands.—In addition to the numerous small glands scattered in the mucosa and underlying connective tissue of the oral cavity, there are in mammals three pairs of salivary glands which arise from the walls of the embryonic oral cavity as epithelial buds. These three pairs of glands, the parotid, submaxillary, and sublingual, display marked histological variation even in closely related species. Their secretions are poured into the mouth as saliva in response to various stimuli, and serve to keep the oral surface moist and to lubricate the food.

Parotid Glands.—Near the base of each external ear is the largest of the salivary glands, the parotid, whose main excretory duct opens into the oral cavity. (Fig. 103.) The lobes and lobules into which the gland is divided are supported by a relatively dense interlobular fibroelastic connective tissue in which a branching system of excretory ducts is located. The largest ducts are lined with a stratified columnar epithelium, and their main branches have a single layer of columnar cells. Adjoining the smallest excretory ducts there are the so-called secretory ducts which occur within the lobules. The secretory portions are composed of columnar epithelial cells with striated basal portions. The secretory ducts divide into intercalated or intermediate ducts, which are very small and formed of cuboidal cells. These terminate in the secreting end-pieces where there are relatively large pyramidal cells of the serous type with rounded nuclei more or less centrally located. The lumens in these secreting end-pieces are very small. The whole field presents a uniform appearance as regards the secreting cells, a condition which is usually not apparent in either of the other salivary glands.

Submaxillary Glands.—This pair of glands is located in the floor of the mouth and the secretion is carried into the oral cavity by an excretory duct which opens on the side of the tongue well forward. The division into lobes and lobules and the arrangement of ducts
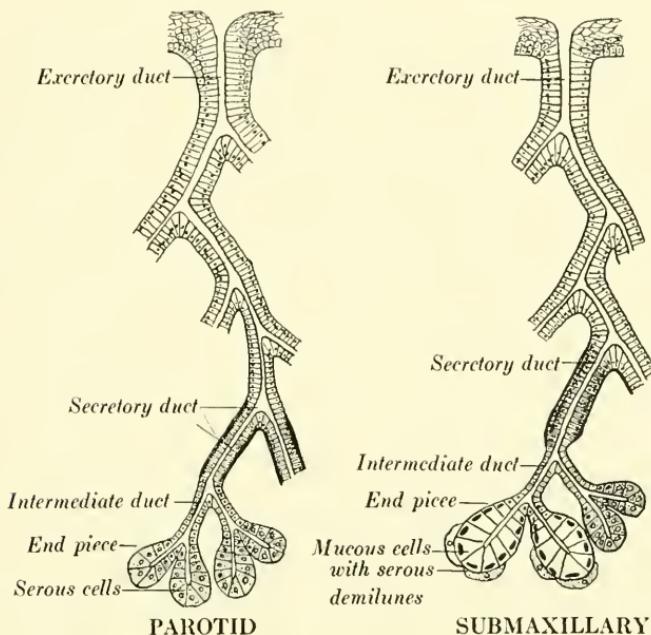
Fig. 103.—Diagrams showing structure of the parotid and submaxillary glands.
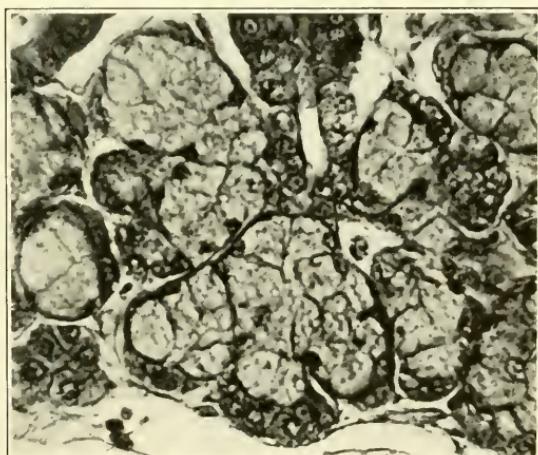
Fig. 104.—Intermediate duct breaking into secretory end-pieces of the submaxillary gland of the cat, showing mucous cells surrounded by crescents of serous cells.

is similar to that found in the parotid. (Fig. 103.) There are two types of cells in the alveoli or secreting end-pieces. A serous type of cell similar to that found in the parotid may be found forming many of the alveoli. Another type of cell is present also, the mucous cell, with lighter, clearer cytoplasm, which takes a basic stain and has basally located nuclei. (Fig. 104.) Outside of some of these mucous cells are crescents of serous cells. The secretory ducts are more extensive in the submaxillary than in the parotid gland. In man, serous cells predominate, but in many other animals, such as the woodchuck and the cat, the mucous cells predominate.

Sublingual Glands.—The sublingual glands are located at the base of the tongue and, like the parotid and submaxillary, are divided into lobes and lobules. The intercalated ducts are lacking as may also be a large proportion of the secretory ducts. Most of the glandular end-pieces (in the cat) are composed of cells of the mucous type, with more numerous crescents of serous cells than in the submaxillary.

The parotid, submaxillary, and sublingual glands are uniformly present only in mammals. There are few, if any, glands associated with the mucous membrane of the mouth cavity of fishes, but mucous glands appear in the oral cavity of Amphibia. In reptiles, mucous glands are located in the mouth cavity near the edge of the jaws, in the tongue, in the wall of the mouth cavity, and also under the tongue. The poison glands of snakes are specialized labial glands. Mucous glands are well represented in this region in herbivorous mammals that feed largely on dry grass.

Pharynx.—The pharynx is posterior to the oral cavity. The pathways of food from the oral cavity to the esophagus, and of air from the nasal passages to the larynx, cross in the pharynx so that this chamber is part of both the digestive and respiratory systems. Embryologically, it is associated with gills, with the trachea and lungs, with the thyroid gland, parathyroid glands, and the thymus, although presenting a great deal of variation in different vertebrates. It is somewhat funnel-shaped, expanded anteriorly and tapered down posteriorly.

THE ALIMENTARY CANAL.

Throughout the alimentary or digestive canal, but not including the mouth or anus, there is a similar structural plan for the wall, which is composed throughout of four coats of tissues.

The innermost coat, the mucous membrane or the tunica mucosa, is composed of the lining epithelium resting on a basement membrane below which is fibroelastic connective tissue called the lamina propria or tunica propria. A thin, double layer of smooth muscle, the muscularis mucosæ, is generally present and marks the external boundary of the mucosa.

Adjoining the mucosa there is a region of looser fibroelastic connective tissue, the tunica submucosa, rich in blood vessels and nerves. The sympathetic nerve plexus carried in this region is called Meissner's or the submucosal plexus. Surrounding the submucosa externally there is a coat of muscle tissue, the tunica muscularis, consisting generally of an inner circular layer and an outer longitudinal layer separated by a narrow region of connective tissue which carries a sympathetic nerve plexus called Auerbach's plexus or the myenteric plexus. The outermost coat of the wall, the tunica adventitia, is composed of fibroelastic connective tissue. This structural plan shows greatest variation in the mucosa of the different regions, especially as regards the glandular development.

The Esophagus.— *The Amphibian Esophagus.*—This part of the digestive tract has the typical four coats. The submucosa extends into a number of long folds carrying the mucosa into the lumen. The epithelium of the mucosa is usually of the stratified ciliated columnar type with numerous mucous cells, although the esophagus of Amblystoma has a mucosal epithelium which resembles the pseudostratified variety, and in Cryptobranchus it is more nearly stratified cuboidal. The muscularis coat is usually represented by a prominent circular sheath, but the longitudinal muscle sheath is in many cases represented by separate bundles. The adventitia connects externally with the skeletal muscles of the neck region. In the submucosa of certain portions of the frog esophagus large compound serous glands occur.

The Reptilian Esophagus.—A study of the esophagus of the lizard (Fig. 105), snake, turtle, and alligator demonstrates that the submucosal folds extending into the lumen are present here also. The mucosal epithelium is in general of the stratified columnar variety. The columnar zone cells may be ciliated and there may be many goblet cells present. The muscularis coat is represented by a distinct circular coat and a longitudinal coat formed by separate strands of smooth muscle cells. The adventitia is of loose connective tissue which supports blood vessels, nerves, and lymphatics.

The Mammalian Esophagus.— Connecting the pharynx with the
stomach is a tube with the characteristic four coats of the digestive
canal already outlined. (Fig. 106.) In the region of the neck the
adventitia consists of loose fibroelastic tissue connecting the esoph-
agus with neighboring structures in the neck. As it passes through
the thorax this outer coat becomes a serosa (visceral peritoneum),
which is composed of an outer thin mesothelial membrane and
an underlying thin layer of connective tissue. The muscularis
may have striated voluntary muscle fibers in the upper region of
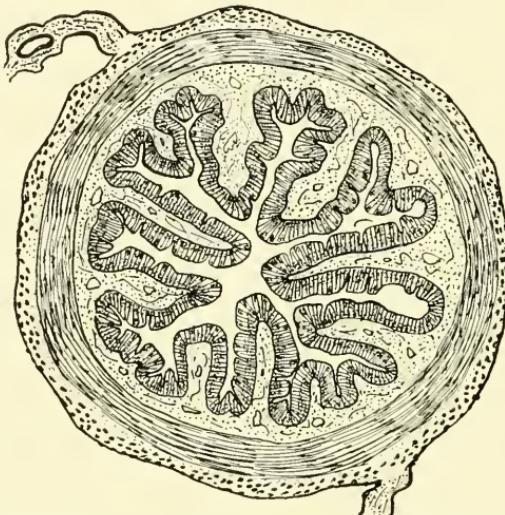
Fig. 105.—Diagram of a cross-section of the esophagus of a lizard. Note the ruga-
like extensions of the submucosa into the lumen. These are covered with stratified
columnar epithelium with many goblet cells. The submucosa supports blood vessels.
The muscularis coat is represented by a well-defined circular sheath. The longi-
tudinal coat is represented by relatively few muscle cells.

the esophagus, a mixture with smooth muscle in the middle region,
and finally a lower portion composed entirely of smooth involuntary
muscle. Its composition is variable, however, for in some animals
striated muscle may be found reaching to the stomach. A very
loose fibroelastic connective tissue forms the submucosa which unites
the muscularis with the mucosa. The submucosa contains large
blood vessels and numerous mucous glands whose ducts open through
the mucosa. A muscularis mucosa marks the beginning of the
mucosa, except in the upper portions of the esophagus where it
may be missing. Within the muscularis mucosa is the fibroelastic
tissue of the tunica propria, which projects as papillæ-like extensions

into the stratified squamous epithelium resting upon it. In addition
to the deep mucous glands found in the submucosa there may also
be simple branched tubular glands lying in the tunica propria and
resembling those to be found in the cardiac portion of the stomach.
When the esophagus is contracted, the mucosa is disposed in longi-
tudinal folds and the lumen is closed.
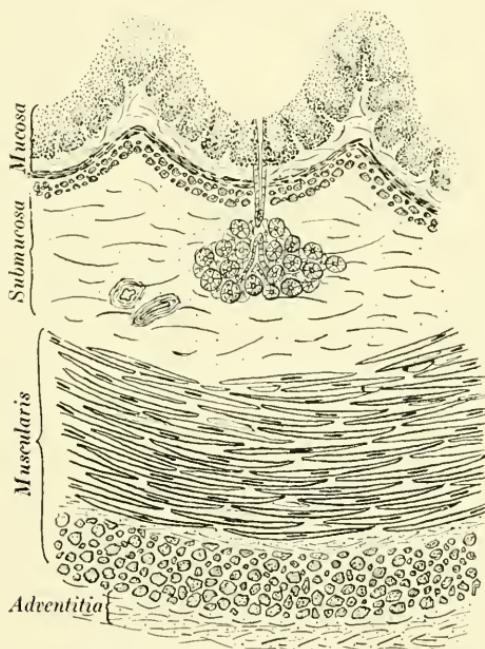
FIG. 106.—Diagram of a cross-section of a mammalian esophagus.

The Stomach.—Beginning with the stomach and extending
through the large intestine, the adventitia is covered by mesothe-
lium, and the coat thus formed is called a serous membrane or
serosa.

The Fish Stomach.—The submucosa has broad ruga-like expan-
sions extending toward the center of the lumen. The mucosa con-
sists of closely packed, slender, simple columnar epithelium lining
the lumen and tubular glands of variable depth. (Fig. 107.) The
peripheral submucosa is not so dense as that adjacent to the epithe-
lium. The muscularis coat consists of a thick circular muscle
sheath and a thin outer longitudinal coat. The adventitia is very
thin. Evaginations occur in the posterior region of the stomach
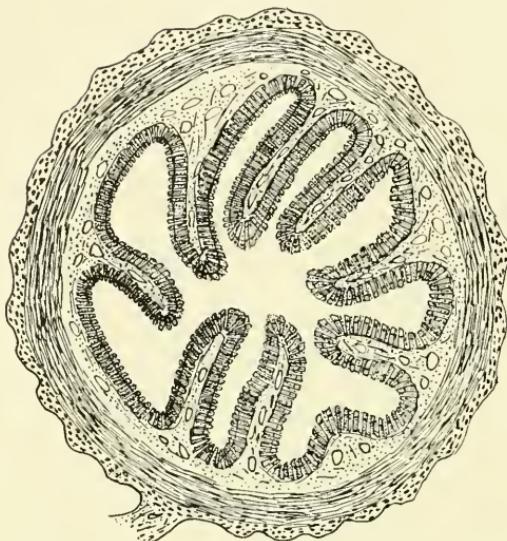
Fig. 107.—Diagram of a cross-section of the stomach of a dogfish (*Squalus acanth-ias*). This shows ruga-like folds of submucosa extending in toward the lumen. The mucosa contains closely arranged simple tubular glands. A muscularis mucosa separates these glands from the submucosa. The muscularis coat is represented by a circular smooth muscle sheath and a thin longitudinal sheath. The adventitia is dense and is associated with the mesentery.
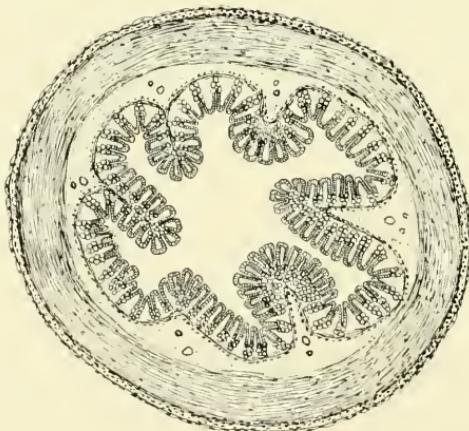
Fig. 108.—A cross-section of the stomach of a frog. The mucosa consists of simple branched tubular glands whose basal secretory cells are serous in character and acidophilic and the cells at the mouth of the gland appear to be mucous and basophilic. There is a well-defined muscularis mucosa. The submucosa is thickest in the region of the ruga-like extensions. The muscularis coat is represented by a thick circular and a thin longitudinal sheath of smooth muscle. The adventitia is a thin serosa.

of teleosts as pyloric ceca, small finger-like pouches structurally similar to the stomach.

The Stomach of Amphibia. — The mucosa consists of simple branched tubular glands lined with simple columnar epithelium and separated from each other by tunica propria. The submucosa has extensions forming folds into the lumen. There is in some species, such as the frog (Fig. 108), a well-defined muscularis mucosa. The muscularis coat has prominent circular muscle and a much thinner longitudinal coat.

The Stomach of Reptiles. — Submucosal folds are present here also. The mucosa is composed of simple tubular glands lined with colum-
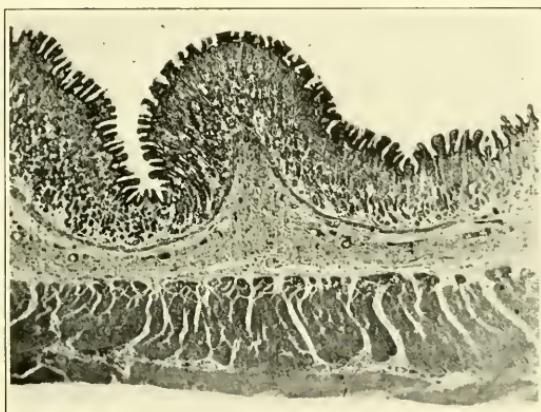
FIG. 109.—Photograph through a ruga of the fundus of the dog's stomach. Note the broad extension of the submucosa surmounted by the glandular mucosa. The muscularis has a thick circular and thinner longitudinal coat. A muscularis mucosa follows the outline of the glandular mucosa.

nar epithelium. The superficial epithelium appears to be stratified columnar in some forms and goblet cells may be numerous. The circular coat of the muscularis is prominent, but the longitudinal coat is much narrower or represented by scattered bundles. The adventia is usually thin.

The Mammalian Stomach. — The mucosa at the lower end of the esophagus is marked by an abrupt transition from a relatively smooth surface and stratified squamous epithelium to the folded and glandular mucosa of the stomach lined by simple columnar epithelium. Below the mucosa the transition is more gradual, the deep mucous glands of the esophagus often extending over into the stomach. Folds in the stomach wall involving submucosa and mucosa form ridges, or rugæ, observed best in the empty stomach.

(Fig. 109.) Small pits, or gastric crypts, are easily visible with slight magnification of the internal mucosal surface. The mucosa is quite thick, due to the presence of simple tubular gastric glands which are roughly divisible into three types: the cardiac, fundic, and pyloric glands.

Cardiac glands, found where the esophagus and the stomach join, are relatively few in number. In these glands the cells are chiefly of the mucous type. Cardiac glands are small, simple branched tubular in form, with a short excretory duct lined by columnar cells. The twisted secretory tubules are formed from cuboidal or columnar cells. There is great variation in the number of cardiac glands, and in some cases they are absent.

FIG. 110. — Diagram of tissues in the body or fundus of stomach of a mammal. A, mucosa with simple, branched tubular glands. The black dots represent parietal cells. Note muscularis mucosa at base. B, submucosa with artery and vein. C, muscularis. This being a longitudinal section, the inner circular muscle is cut across and the outer longitudinal muscle is cut lengthwise. D, adventitia, which is a serosa with an external limiting membrane of mesothelium.

Fundic glands are most numerous and produce the essential elements of gastric juice, the cardiac and pyloric glands may function mainly as mucous glands. The fundic glands are branched tubular in form, with a relatively short excretory portion extending into the gastric pit, and glandular portions that are generally very much longer. (Fig. 110.) The cells of the gastric pit walls are columnar in type. A constricted portion of the gland near the gastric pit is called the neck, and from it each secretory tubule leads to a dilated end. Two types of cells, the chief or central cells and the parietal cells make up the secreting tubules. The chief cells are roughly pyramidal, their cytoplasm shows secretion granules in the end toward the lumen, and nuclei are located in the basal half. The secretory activity of these cells is associated with the production of zymogen granules, which give rise to the pepsin of the gastric juice. Scattered along the secreting tubule, between the chief cells and the basement membrane, and more numerous toward the neck, are the parietal cells
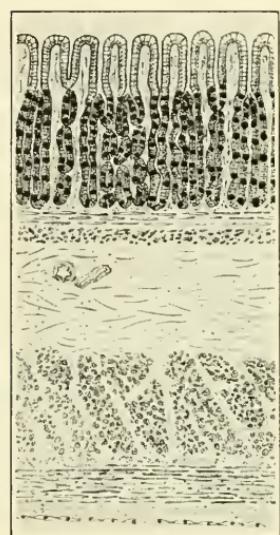
which are larger than the chief cells. These cells are oval or polygonal, their finely granular cytoplasm has an affinity for acid dyes, and the large spherical nucleus is centrally located. Parietal cells are associated with the production of the hydrochloric acid present in the gastric juice, and are often called oxyntic or acid cells for this reason.

The pyloric glands intermingle with the fundic type in that portion of the stomach near the small intestine. The transition is gradual; parietal cells become less and less numerous and finally no longer appear in the more typical pyloric glands. The gastric pits become longer, and the secreting tubules become relatively shorter until they are about as long as the excretory portions but are more twisted than in the fundic region. The cells of the pyloric glands are distinctly mucous in appearance.

The tunica propria of the stomach extends in between and around the secreting tubules of the gastric glands, and the muscularis mucosa lies just below the deepest ends of the secreting tubules. Scattered diffusely throughout the tunica propria are lymphocytes, but in some regions solitary lymph nodules occur. The submucosa is typical, being composed of loose fibroelastic connective tissue whose longitudinal ridge-like extensions form rugæ. The muscularis may have three layers in some regions, an inner oblique, a middle circular, and an outer longitudinal layer. In the pyloric region the two inner layers are thickened to form a sphincter muscle. The adventitia is composed of a coat of loose fibroelastic connective tissue enclosed by a single layer of mesothelium.

Although the stomach functions mainly as a temporary place for storing food, some digestion occurs as a result of glandular action. Proteins may be converted into proteoses and peptones by the action of pepsin, while another enzyme, rennin, if present in the gastric juice, plays a rôle in the digestion of milk.

The Small Intestine.—Continuing from the stomach, the digestive tract becomes a smaller tube whose internal surface is much increased by numerous folds. The muscularis coats, both the circular and longitudinal, tend to encircle the tract in a gentle spiral. In general, with the exception of the birds and mammals, the glands that are a prominent feature in the stomach mucosa are lacking in the intestine where the main function is absorption of the material digested by the secretions passing into it from the stomach and from the pancreas and liver. The epithelium of the mucosa may contain numerous mucous secreting cells which cover the membrane with their lubricating secretion.

The Small Intestine of Fishes.—In the small intestine of the dog-
fish (young *Squalus acanthus*) several submucosal folds extend into
the lumen. (Fig. 111.) The mucosa is composed of columnar
epithelial cells which rest upon a tunica propria which, due to the
absence of a distinct muscularis mucosa, is continuous with the
submucosa. Folds of the mucosa form tubular pockets at the base
of the folds; these resemble simple tubular glands but their cells
show no special secretory activity differentiating them from the
other cells of the mucosa. The muscularis coat has a broad circular
region but the longitudinal portion is poorly represented. The
connective tissue of the adventitia is covered by cuboidal mesothe-
lial cells.
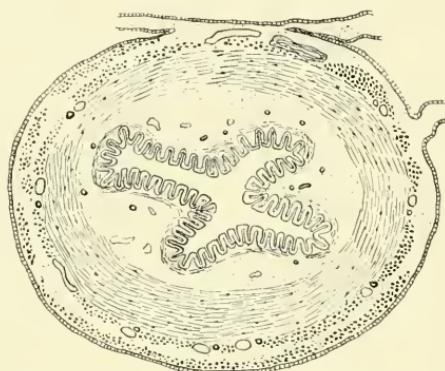
Fig. 111.—Diagram of a cross-section of the duodenum of a dogfish. The sub-
mucosa has ruga-like extensions into the lumen. The mucosa consists of simple
tubular glands lined with columnar epithelium and a thin muscularis mucosa. The
submucosa is thickest in the region of the ruga-like extensions. The muscularis is
represented by a thick circular coat and a thin longitudinal coat which is present in
separate strands. The adventitia is a serosa covered with cuboidal epithelium.

Among the teleosts the structural plan is similar to that in the
dogfish but the mucosal folds appear much more elaborate and the
epithelial membrane appears to be commonly composed of stratified
or pseudostratified columnar cells.

The Small Intestine of Amphibia.—There are wide, longitudinal
submucosal ridges in the intestine of urodeles. In Necturus, the
epithelium is simple columnar in type, with many goblet cells
present in it. There is diffuse lymphoid tissue in the submucosa.
The circular muscle coat of the muscularis is thicker than
the longitudinal coat. Superficially, mesothelium covers the ad-
ventitia. In the frog (Fig. 112), numerous small folds com-
posed of mucosa and a core of connective tissue extend into the

lumen. Larger folds extend out from the submucosa carrying along these smaller folds. The submucosa does not form the wide ruga-like extensions, as in the urodeles, but it is present between the adjacent epithelial surfaces of the villus-like folds. The circular coat of the muscularis is not very thick, and the longitudinal coat is still thinner.

The Reptilian Small Intestine.—In reptiles there are numerous longitudinal folds of the mucosa and submucosa. The muscularis mucosa may be present as a scattered region of circular cells, or the submucosa and tunica propria may form a single narrow connective-tissue region. The circular layer of the muscularis is the thickest
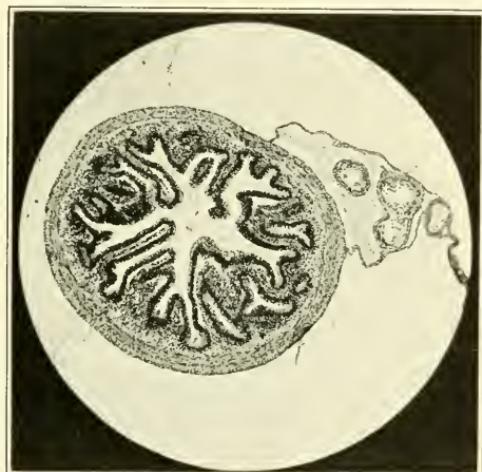
Fig. 112.—Photograph of a cross-section of the small intestine of the frog, showing villus-like longitudinal folds. Bloodvessels are shown in the supporting mesentery.

coat; the longitudinal coat is very thin. Mesothelium covers the thin adventitia.

In the lower vertebrates the intestine shows little or no marked histological differentiation at the various levels, but in the anterior portion the submucosal folds are more numerous, the lumen smaller, and the wall proportionally thicker.

The Small Intestine of Mammals.—The same four coats noted in the stomach form the wall of the small intestine, but here the muscularis is quite regular, with an outer longitudinal and an inner circular layer. (Fig. 113.) The mucosa possesses characteristic features that deserve closer attention. The inner surface of the canal generally shows circular and oblique folds involving the

mucosa and part of the submucosa. These folds, paralleling each
other and extending part way around the lumen at irregular inter-
vals, are called valvulæ conniventes, or plicæ circulares. The
mucosa is modified by extension into the lumen of papilla-like pro-
jections which may be leaf-like, finger-like, or broadly club-shaped.
These structures are known as villi and are diagnostic features of
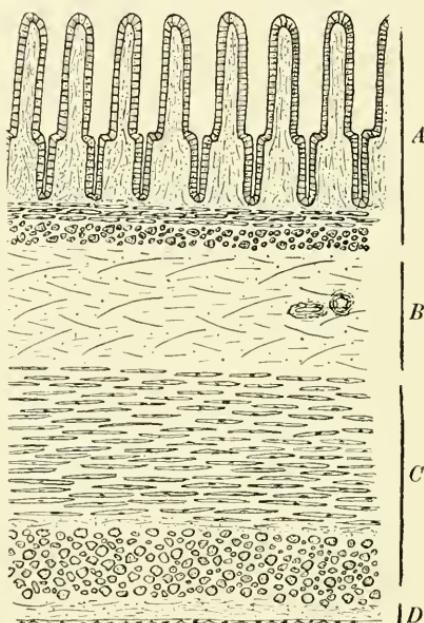
Fig. 113.—Diagram of tissues in the wall of the small intestine of a mammal.
A, mucosa showing villi with the glands of Lieberkühn extending basally between
them. At the base of these glands is the muscularis mucosa. B, submucosa with
an artery and vein. C, muscularis coat with an inner circular and an external
longitudinal muscle coat. D, adventitia with its external limiting membrane of
mesothelium.

the small intestine of mammals. They serve to increase the surface
and function in the process of absorption.

The villi in a relaxed condition of the tract nearly fill the lumen.
Each villus has a core of the loose fibroelastic tissue from the tunica
propria and scattered smooth muscle cells from the muscularis
mucosa. Simple columnar epithelium with basally located nuclei
cover the villi. The cells have a striated cuticular border, the
striated appearance being claimed by some observers to be due to
minute canaliculi through which absorption of digested material
is effected. (Fig. 114.) Scattered among the simple columnar cells

are goblet cells. These appear in varying numbers and become more markedly abundant in the ileum. Within the epithelium of each villus there is loose connective tissue, a basketwork of blood capillaries, a nerve net, and some diffuse lymphoid tissue. A dilated blind lymph capillary, a lacteal, occupies the central portion. The villi vary in shape, size, and number in various mammals. From the bases of the villi extend simple tubular glands which formed as invaginations of the embryo gut epithelium. These are called Lieberkühn glands and extend down to the muscularis mucosa. The epithelium lining them is continuous with that covering the
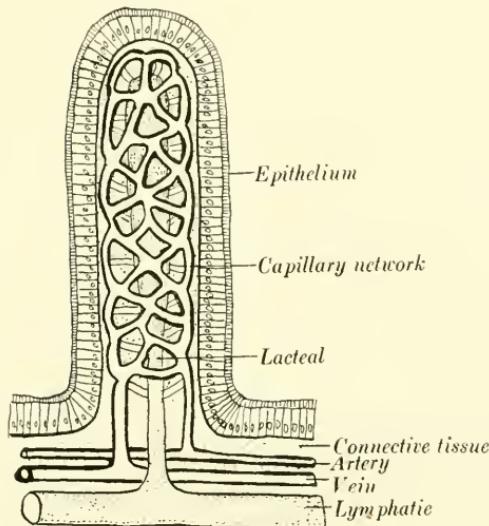
Fig. 114.—Diagram of a villus.

villus, but the cells are shorter and have no striated border. Near the base of these glands occur coarsely granular, basally striated cells, the cells of Paneth, which secrete a serous fluid containing an enzyme. These cells are widely distributed throughout the epithelium of the digestive canal of higher vertebrates. Another type, which reduces silver, is called an argentaffin cell and has a finely granular basal portion of cytoplasm. It has been demonstrated scattered among the cells lining the crypts and rarely among those covering the villi, but as yet its nature is not fully understood. The tunica propria, or stroma, as it may be called, packs in between the Lieberkühn glands, as well as forming the core of the villi. Its light, loose reticular network and denser fibroelastic tissue supports

12

a diffuse lymphoid tissue, and solitary lymph nodules are relatively common. In the ileum, near the jejunum or lower, the lymph nodules form groups, called Peyer's patches, which may extend into the submucosa. No villi cover some nodules which project into the lumen. The muscularis mucosa may be of varying distinctness with an outer longitudinal and inner circular layer of smooth muscle.

The submucosa of loose fibroelastic tissue carries the larger blood and lymph vessels, Meissner's nerve plexus and, except in the region of the duodenum, has no glands. Branched tubular glands, Brunner's glands, appear in the submucosa of the upper region of the duodenum and often extend over into the adjoining region of the pyloric stomach. The secretory portion of these glands is composed of pyramidal or columnar epithelium. The secretion contains a proteolytic enzyme similar to pepsin in its action. Ducts from these glands lead up through the mucosa and open either between the villi or into the crypts of Lieberkühn glands. The cells lining the excretory ducts are similar to those of the epithelium lining the duodenum.

Outside the submucosa is the muscularis coat consisting of an inner sheath of circularly disposed smooth muscle and an outer longitudinal coat. Connective tissue containing Auerbach's plexus lies between them.

The adventitia is composed of a thin layer of fibroelastic connective tissue covered with mesothelium.

The Large Intestine. — *The Large Intestine of Elasmobranchs.* —The large intestine of Selachians consists of the so-called spiral valve, in which the inner surface of the tube is a spiral membranous shelf formed by an extension of submucosa covered with a mucosa. An adventitia with superficial cuboidal epithelium forms the external coat. Internal to this are scattered strands of circularly arranged smooth muscle cells, forming a circular coat of a muscularis. The submucosa of fibroelastic connective tissue forms the core of the spiral valve. The mucosa has a number of simple tubular involutions resembling glands but they are lined with a stratified or pseudo-stratified columnar epithelium. The epithelium covering the mucosa not so invaginated is of the same type as that found between the folds. The folds help to increase the absorption of material passing over the valve but have no secretory activity. Lymphoid and myeloid tissue may be conspicuous in the connective tissue below the epithelium.

In part of the intestine below the spiral valve in the Selachians, and over the entire lower portion in those forms not having a spiral valve, the structure resembles that of the small intestine. The mucosa becomes smoother due to loss of mucosal and submucosal folds.

The rectal gland of the Selachian is a short cylindrical structure attaching to the colon just posterior to the end of the spiral valve. A study of sections at various levels shows that it is a compound tubular gland. The tubular secretory end-pieces are composed of cuboidal cells apparently serous in nature. The function of the gland is not definitely known.

The Large Intestine of Amphibia.—The mucosa is thrown into several longitudinal folds by the submucosa. The epithelial lining is stratified or pseudostratified columnar with numerous goblet cells. Small glands may extend a short distance into the tunica propria. The muscularis has an inner circular coat and an outer longitudinal coat, but the adventitia is reduced to a very thin membrane of connective tissue covered with simple squamous epithelium. In the frog, seromucous glands occur in the tunica propria, which may be separated from the submucosa by a thin circular muscularis mucosa.

The Large Intestine of Reptiles.—The mucosa has simple tubular glands that extend to a muscularis mucosa. The glands are lined with simple columnar cells among which are goblet cells. Diffuse lymphoid tissue is quite abundant in the submucosal tissue. The muscularis has a definite inner circular coat and a thin outer longitudinal muscular sheath. Extending lengthwise are branching submucosal folds which appear like long villi when the intestine is studied in sections.

The Mammalian Large Intestine.—The same four coats make up the wall here as observed in the case of the small intestine, but certain characteristic modifications of the mucosa distinguish it. (Fig. 115.) There are no villi and generally no plicæ circulares present. The mucosa has many tubular glands extending to the muscularis mucosa. These glands are homologous with the glands of Lieberkühn of the small intestine, but are longer. The cells lining the surface of the lumen are tall columnar and may have a thin striated cuticular border. Passing down into the glands, the cells become shorter and goblet cells become more numerous. Goblet cells are most numerous in the mid-region of the glands. The basal cells are less differentiated and are supposed at

times to give rise to cells replacing worn-out goblet cells. The
goblet cells of glands secrete mucus to lubricate the intestinal
lumen surface, and thus aid in the passage of the fecal material
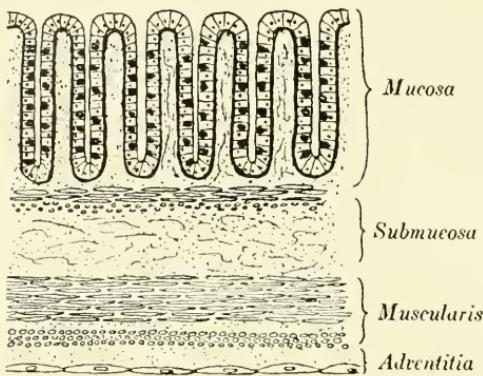
FIG. 115.—Diagram of large intestine of mammals.

which becomes more solid, due to absorption of water through the
intestinal wall.

The wall of the cecum usually has lymph nodules in the sub-
mucosa. In some forms, as in the rabbit, these nodules may encircle
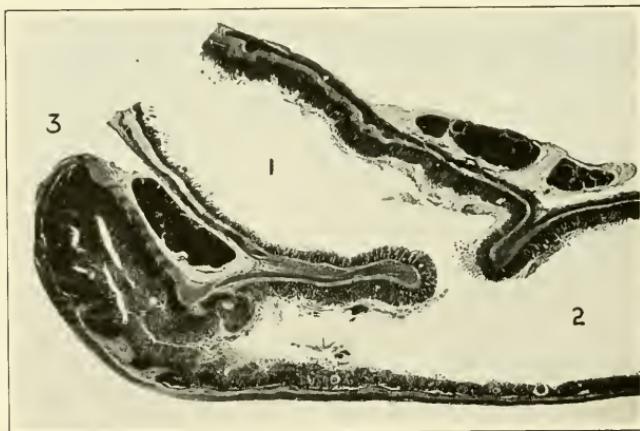
FIG. 116.—Photograph of the ileocecal junction of the cat, showing the valve and
the appendix, *3; 1*, ileum; *2*, cecum.

the lumen and form the greater part of the wall with a reduction in
amount of connective tissue and muscularis.

The vermiform appendix is a blind pouch extending from the end
of the cecum at its junction with the small intestine. (Fig. 116.)

The wall has a mucosa with Lieberkühn glands. The characteristic feature is the abundance of lymphoid tissue which is present, not only as nodules in the mucosa and submucosa, but also as diffuse tissue throughout the tunica propria. The circular coat of the muscularis is well represented, but the outer longitudinal coat is thin. In some cases the glands may be obliterated by lymphoid tissue and the lumen filled with lymphocytes that penetrate through the mucosa.

The Rectum and Anus of Mammals.—The rectum has long and large tubular glands and solitary lymph nodules are common in its mucosa and submucosa. Longitudinal folds of the mucosa appear in the lower part of the rectum, and with them occurs a change in the character of the mucosa. The columnar epithelium and simple tubular glands of the rectum change abruptly to stratified squamous epithelium of the anus, and this epithelium is continuous with the skin in much the same fashion as already noted in the case of the lip at the other end of the digestive tract. The muscularis of the rectum has two complete layers, and near the anus striated muscle replaces the smooth muscle.

The Cloaca.—In vertebrates below mammals the rectum and uro-genital ducts open into a common chamber, the cloaca. In addition to being a mere receptacle it shows modifications for transmission of genital products and there is considerable variation in its structure. In fishes it is lined with stratified squamous epithelium supported by dense fibroelastic connective tissue and some muscle. In Amphibia it may be a sac lined with columnar cells, among which are numerous goblet cells, or it may have glandular linings associated with reproductive activities. Among the reptiles and birds, the ventral wall of the cloaca may be developed into organs for copulation and portions of the wall may be glandular.

The anus or cloacal opening is lined with stratified epithelium similar to that of the skin and is supported by loose fibroelastic connective tissue joining with the muscles underlying the skin of this region.

THE PANCREAS.

The pancreas has been called the salivary gland of the abdomen, and at first glance resembles a parotid (see Parotid) in the serous appearance of its secretory cells. It is a compound tubulo-alveolar gland lying behind the stomach and attaching to the duodenal wall. No distinct capsule covers it, and its lobules are separated by a

loose fibroelastic tissue. The secreting end-pieces, which may be
tubular or short and roughly spherical, are formed of pyramidal cells.
The cytoplasm of these cells is lighter staining toward the lumen
and more deeply staining with striated appearance in the basal
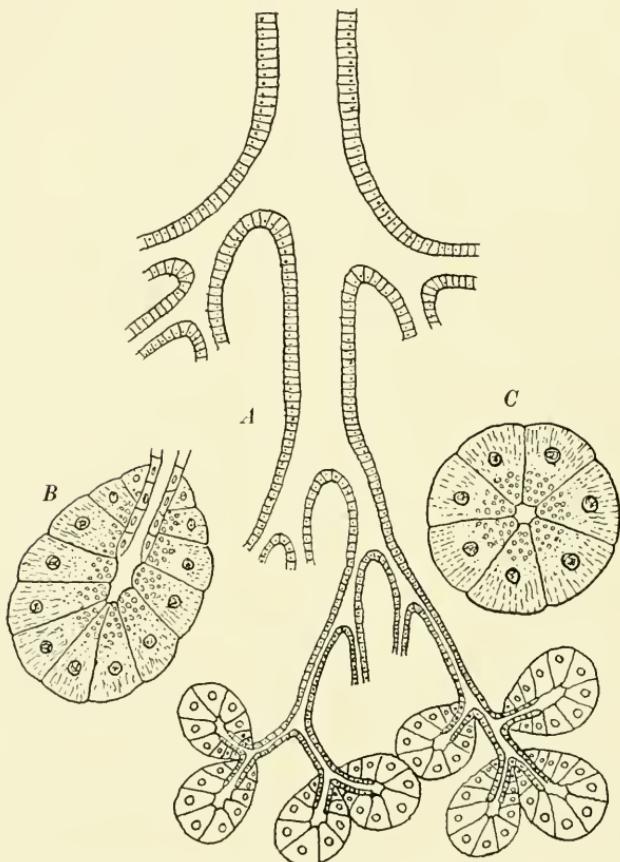
FIG. 117.—Diagrams showing structure of pancreas. *A*, a small excretory duct
with its branches terminating in secretory end pieces. The intercalated ducts
extend part way into the end-pieces. *B*, longitudinal section of an end-piece, showing
centro-acinar cells (involuted intercalated duct) and the secretory cells which have
basal striations and secretory granules toward the lumen. The secretory cells are
serous in type. *C*, cross-section of a secretory end-piece distal to centro-acinar cells.

region. (Fig. 117.) The lighter portion contains coarse zymogen
granules, which are presumably forerunners of the enzymatic secre-
tions. In the basal half of the cell there is a nucleus with a chro-
matin network and one or more nucleoli. In sections, centro-
acinar cells may occupy the lumen of many acini along the inner ends

ends of the secreting cells. These cells represent a continuation
of the intercalated ducts which project a short way into the acinar
lumen. Such cells have no secretory granules and possess a small
deeply staining nucleus. Intercalated ducts lined with first cuboidal
then low columnar pass to small excretory ducts lined with a tall
columnar epithelium. The large excretory ducts are lined with
a stratified columnar epithelium and pass into the main duct (duct
of Wirsung), which opens into the intestinal lumen. Enzymes
(trypsin, amylase, and lipase) present in the pancreatic secretion
break down the proteins, starches, and fats into simpler compounds.
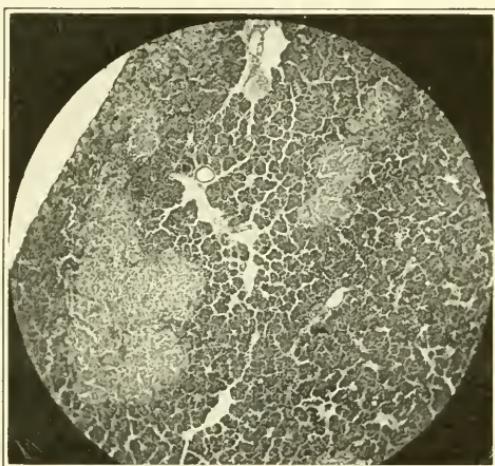
FIG. 118.—Section of pancreas of the water snake (Natrix). It is similar to the
pancreas of a mammal but relatively less connective tissue makes it more compactly
glandular. The light gray areas are sections of islands of Langerhans.

Though at first glance it resembles the parotid of mammals, the
pancreas differs in the nature of its secretory cells, in the looser
arrangement of the interlobular connective tissue, in the absence
of secretory ducts, and finally in the presence of certain groups of
very distinct lightly staining cells forming the islands of Langerhans.

The islands of Langerhans are composed of spherical groups of
from few to hundreds of lightly staining cells irregularly scattered
among the acini and along the ducts. (Fig. 118.) These cells are
arranged in cords forming a network with numerous wide capillaries
passing between the cords. Two types of cells are demonstrated on
the basis of secretory granules which are not in either case like those
of the acinar cells of the pancreas. One, the A cell, with fine

cytoplasmic granules and a large oval nucleus with little chromatin apparent is found most frequently in the center of the island. The second type, the B cell, is smaller and more numerous, with a smaller nucleus containing large chromatin granules. The secretion of these island cells, insulin, passes into the blood and governs the metabolism of carbohydrates. The appearance of these islands may vary considerably, even occurring as a separate gland outside the pancreas in some fishes.

In lower vertebrates the pancreas is usually a more compact gland, but in general closely resembles the mammalian structure just described. Intercalated ducts are usually not present and centro-acinar cells are absent.

THE LIVER.

The liver arises as a diverticulum of the mid-gut and develops into a compound tubular gland. Among fishes it may retain this simple glandular condition with blindly ending secretory tubules. With the amphibia and reptiles the tubules fuse to form a network and the liver cells surround a central lumen into which secretions are poured from bile capillaries which form as a network of grooves between the adjacent faces of the cells. The connective tissue is not abundant, and no lobulation occurs. In mammals there is an increase in amount of connective tissue and lobulations are indicated where connective tissue accompanies the larger blood-vessels. Among the mammals the lumens of the embryonic end-pieces of the gland are lost, so that only cords of cells are apparent and an increasing amount of connective tissue results in a division of the gland into lobules, and groups of these form lobes.

Lower Vertebrates.—The liver of fishes is a typical compound tubular gland with the clear cells of the secreting end-pieces arranged about the lumen into which the secretion collects. Small bile ducts are formed of cuboidal cells. Between adjacent end-pieces and ducts and lobular masses is a small amount of connective tissue which supports capillaries and small vessels. Another type of cell with granular pigmented cytoplasm often occurs around the larger excretory ducts and bloodvessels.

The liver of amphibians is a modified compound tubular gland. (Fig. 119.) The peripheral end-pieces composed of the secreting cells form a series of differently directed short tubules that connect and form a network. The cells are large and pyramidal shaped,

with clear or vacuolated cytoplasm. The smaller bile ducts are of
low cuboidal cells that have the same embryonic origin as the
secreting cells. Between adjacent end-pieces is a small amount of
connective tissue supporting sinusoids similar to those of the mam-
malian liver. Among the secreting cells are groups of pigment
cells. The bile ducts are at first composed of cuboidal cells, the
larger branches are formed of columnar cells, and the still larger
branches have stratified columnar epithelium. In the Urodeles,
the tissue of the inner zone of the capsule contains hemocytoblasts
and neutrophil and eosinophil myelocytes, and acts as a hemapoietic
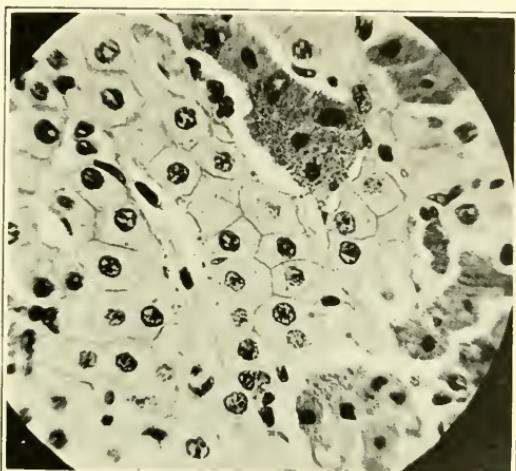
Fig. 119.—Section of liver of Amblystoma, showing rows of large polyhedral hepatic
cells separated by sinusoids. Cells filled with pigment are also shown.

center. The mammalian liver has this property only during early
embryonic life.

The reptilian liver has the appearance of a compound tubular
gland also. The secreting end-pieces are composed of large poly-
hedral cells. The bile ducts resemble in general those of a mam-
malian salivary gland. Between adjacent tubules is a prominent
capillary network. In the connective tissue between lobules can
be found the so-called portal canals, *i. e.*, an artery, a vein, and a
bile duct.

The Mammalian Liver.—This is the largest gland in the body and
lies below the diaphragm in the upper region of the abdominal cavity.
The fibroelastic connective sheath, or capsule of Glisson, completely
surrounds the liver. At the hilum, where the blood vessels enter

the connective tissue of the capsule, tissue of the same sort as the latter continues inward, accompanying the vessels, and divides the liver into several lobes, which in turn are subdivided into numerous lobules. (Fig. 120.) The liver lobule is the unit of hepatic structure in mammals, each lobule being roughly a five- or six-sided polyhedral prism in form. Each lobule is composed of anastomosing cords of liver cells that radiate outward from the center which is occupied by a central vein. The individual cords have two rows of cells whose boundaries are normally clearly defined. The individual cells are polyhedral in form, with a central nucleus, or possibly two or more nuclei, each with one or more nucleoli. Granules of glycogen (animal starch) may be demonstrated in the
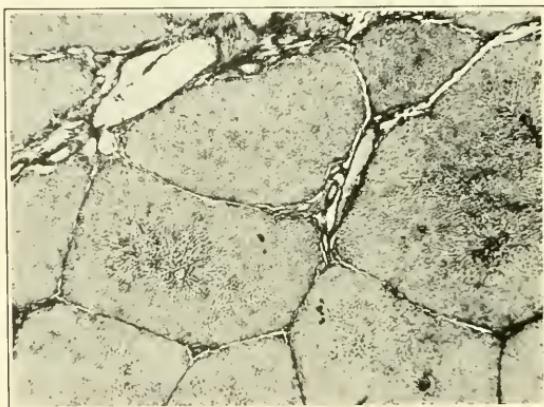
Fig. 120.—Photograph of several lobules in the liver of the pig.

cytoplasm. Fat droplets appear to be normally present and vary inversely in amount with the glycogen. Pigment granules may also be present. Considering the variety of functions in which the hepatic cells participate, it is surprising that there is only one type of cell. Instead of the cells pouring their secretions into a lumen which the free ends of the cells adjoin, as is the case in other exocrine glands, the hepatic cords have a network of small canaliculi, the bile capillaries, running between adjacent faces of cells forming the cords.

To understand the composition of the liver it is important to understand the distribution of the blood vessels to a lobule. Blood enters the liver from two sources, the hepatic artery and the portal vein, and leaves through the hepatic veins which empty into the vena cava. The afferent vessels, the hepatic artery and portal

vein, enter at the hilum and divide into large interlobar branches
following the septa separating the lobes. The interlobar vessels
give off the interlobular branches which follow the septa between
the lobules. The interlobular branches of the portal veins give off
short branches passing to the surface of the lobules where they
break up into an intralobular capillary network. (Fig. 121.) The
hepatic artery follows the portal vein in its branching to the inter-
lobular septa, where its finer branches break up into capillary net-
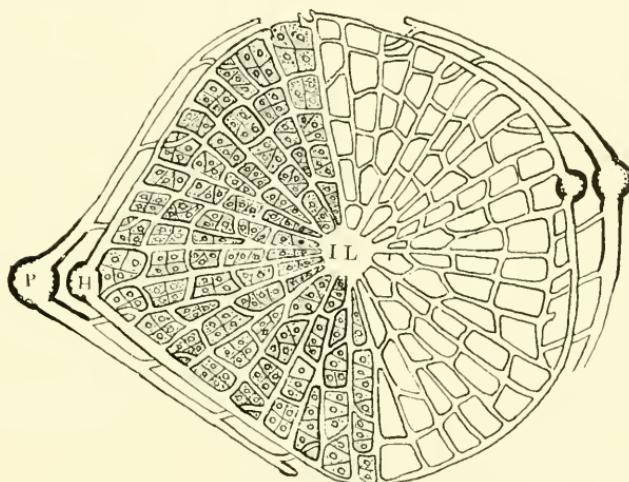
FIG. 121.—Diagram of a cross-section of a lobule of the liver of a mammal with
the hepatic cells radiating toward a common center. *P*, section of a branch of the
portal vein in the interlobular connective tissue; *H*, branches of the hepatic artery
near the branch of the portal vein; branches of this portal component extend to form
the capillary system of the lobule. Some twigs of the hepatic artery components
also join this capillary system. The diagram shows the capillaries forming a mesh-
work among the cords of liver cells. The capillaries converge toward the intralobular
vein (*IL*) in the center of the lobule.

works, some of which supply the septa and then join the finer
branches of the hepatic vein, while others enter the lobules to join
with the intralobular capillary system.

The intralobular capillary network is composed of wide lumened,
hepatic sinusoids. These anastomose irregularly along the free
surfaces of the cords of hepatic cells and everywhere separate the
cords from each other, so that each liver cell has several of these
sinusoids in contact with it, a disposition not found in the case of
other gland cells. The sinusoids carry the blood toward the center
of each lobule, where they empty it into the central vein which
emerges at the base of the lobule. Each central vein joins a sub-

lobular vein which eventually unites with the hepatic veins, the efferent blood vessels of the liver. In addition to the endothelial cell proper, which is small and flat with a darkly staining nucleus, there are present along the walls of the sinusoids other larger cells, the Kupffer cells. Kupffer cells have a number of cytoplasmic processes and a larger vesicular nucleus. By intravenous injection the Kupffer cells can be shown to be active in ingesting foreign particles from the blood passing through the sinusoids, *i. e.*, they become macrophages. They may be considered as homologous with histiocyte cells and are part of the reticulo-endothelial system. Some believe they are derived from the cells of the delicate reticular
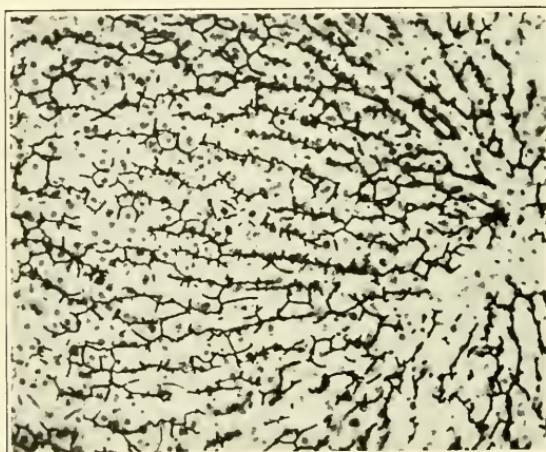
Fig. 122.—Photograph of injected bile capillaries in the liver of a rabbit. These are indicated by the network in black. Nuclei of liver cells are faintly stained and the cord-like arrangement of liver cells is evident.

tissue enveloping the sinusoids; others consider them derived from endothelial cells. Possibly both sources are correct.

Histiocytes or reticulocytes and endothelial cells, forming the sinusoids in the spleen and marrow, and Kupffer cells are considered homologous and constitute the reticulo-endothelial system, important physiologically because of its phagocytic potentialities.

Between the adjacent faces of the hepatic cells the bile capillaries appear as grooves between the adjoining cell walls. By silver impregnation and injection the bile capillaries (Fig. 122) appear to be bounded by a delicate non-cellular membrane formed by the adjoining hepatic cell walls. These capillaries are functionally comparable to the lumens of other glands, and into them is collected

the bile secreted by the hepatic cells. Each hepatic cell may have more than one bile capillary into which it discharges bile, but they occur only between adjoining surfaces of cells and not along the free surfaces which adjoin the blood sinusoids. These capillaries form a branching network and join those of adjoining hepatic cords to carry the bile outward toward the periphery of the lobule. At the periphery of the lobule they collect into small bile ducts in the interlobular septa. These interlobular bile ducts have walls formed of flat or cuboidal epithelium and coalesce to form larger ducts following the course of the portal vein and hepatic artery through the septa. With increasing size of the bile vessels, the epithelium of their wall changes to tall columnar which is surrounded by a coat of connective tissue and scattered longitudinal smooth muscle cells. The larger bile ducts from each lobe empty into a larger hepatic duct that in turn joins the common bile duct leading into the duodenum.

The three vessels coursing close together through the connective-tissue septa of the liver, i. e., a branch of the hepatic artery, a branch of the portal vein, and a bile duct, form the so-called portal canal peculiar to the liver. The vein is the largest, the bile duct is second in size, and the artery is the smallest vessel of the three.

As a gland the liver is peculiar in passing its secretions peripherally to the collecting ducts and, although the hepatic cells are only of one type, they are presumably equally capable of many different functions. Moreover, the blood courses not outward from a center, but in toward the center of each lobule where it is collected into efferent veins that follow at first a course different from the small afferent vessels and ducts of the gland. The amount of connective tissue varies in different animals, being particularly well represented in the pig liver, where the interlobular connective tissue clearly marks the boundaries of the lobules. The arrangement of the hepatic cells is unique as regards their relation to sinusoids, and the bile capillaries are mere troughs between the adjacent faces of two cells.

The liver is associated with the production of bile which is carried to the duodenum, where it is mixed with food and pancreatic enzymes. Part of the bile consists in secretion products from the liver cells, and in part it consists of excretions which the liver cells have removed from the blood. Apparently liver cells are able to excrete foreign substances that have entered the blood. Bile acids produced by the liver cells take part in the absorption of fats from

the intestine. The liver also has important endocrine functions. It forms urea from ammonium carbonate and later on the urea is removed from the blood by the kidney and excreted. The most important endocrine function of liver cells involves taking such a simple carbohydrate compound as glucose from the blood and changing it to temporarily insoluble glycogen. Then as tissues, especially the muscles, need this fuel the liver reconverts glycogen back into simple sugar (glucose) in which form it is distributed by the blood-vascular system. The presence of insulin derived from islands of Langerhans in the pancreas is essential to the carrying out of this function. Tests show that liver cells, depending on the diet, in some cases show a preponderance of protein products, at other times of glycogen, and at still other times of fats. An extract, called heparin, obtained from liver tissue prevents the clotting of blood. Another substance obtained from liver tissue stimulates the production of erythrocytes. Liver tissue possesses great power of regeneration, but the removal of the entire organ results in the death of the organism in a short time.

THE GALL-BLADDER.

The largest bile ducts from the lobes of the mammalian liver usually connect with a pouch, the gall-bladder, which lies along the posterior under surface of the liver. The cystic duct continues outward to join the common bile duct. The wall of the gall-bladder consists of three coats, a mucosa, muscularis, and an adventitia. The mucosa is much folded and has a covering layer of tall columnar epithelial cells with basal nuclei. The tunica propria carries blood vessels, and toward the neck region of the pouch there occur small tubulo-alveolar glands. The muscularis possesses interwoven bundles of smooth muscles arranged as an inner longitudinal layer and an outer thicker circular coat. The outer part of the adventitia is not so dense as the inner and supports blood and lymph vessels and nerves.

In the fish, if present, it has a lining of simple or stratified columnar epithelium surrounded by fibrous connective tissue. In the frog, it is lined with columnar epithelium surrounded by connective tissue which supports blood vessels. In the water snake (Natrix), it is lined with stratified columnar epithelium, and outside this is connective tissue with smooth muscle cells in it disposed in strands around the wall.

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See Appendix for general text references.

CHAPTER XII.

THE EXCRETORY SYSTEM.

THE oxidation phenomena grouped under metabolism, and involving both synthesis and destruction of cell materials, are accompanied by the production of waste products. Such waste products include carbon dioxide, mineral salts, water, and numerous simple nitrogenous compounds. These are mainly removed from the organism in aqueous solutions. We have already observed that in vertebrates carbon dioxide and some water are removed from the blood through the respiratory system, and that the integument plays a part in the elimination of small amounts of waste. However, among most animals a distinct excretory system has been evolved to deal with nitrogenous wastes.

In the chordates we find the presence of a segmental condition in the excretory system. In the primitive forms there is a system of segmental nephridial tubules with ciliated openings (nephrostomes) into the cœlom and outlets direct to the exterior. With the cephalochords such tubules have nephrostomes in the cœlomic cavity and are also associated with coils of blood vessels, so that wastes are removed from both the cœlomic fluid and the blood. With the vertebrates there is an increasing efficiency in the development of nephridial tubules for the removal of wastes from the blood, and they become organized into one of three types of excretory organs called kidneys. These form in the embryo along the mid-dorsal region, one on each side, at different levels. The three types have a common source of origin in the mesoderm of the intermediate cell mass, the nephrotome, which lies lateral to the mesodermal segments and connects them with the somatic and splanchnic layers of mesoderm enclosing the cœlom. Tubules formed from this mesodermal region become grouped along the mid-dorsal region to form each type of kidney.

In the cyclostomes, young fish, and young amphibians, the *pronephros*, or head kidney, is functional; in adult fishes and amphibians, the *mesonephros*, or middle kidney, is functional; in reptiles, birds, and mammals, the *metanephros*, or posterior kidney, becomes

the functional organ. During the embryology of those forms having the metanephros, the other two types are formed successively and may function temporarily until the metanephros takes over the excretory function.

THE PRONEPHROS.

The pronephros consists of short, paired segmental tubules located well toward the head region, the tubules of each side connecting with a longitudinal duct on that side, and this opens posteriorly into the cloaca. Each pronephric tubule has a proximal, ciliated, funnel-like end, the nephrostome, opening into the cœlom. As they develop, the distal portion of each tubule bends backward and unites with the distal end of the next tubule to form the common longitudinal collecting duct, the pronephric duct, for each side. As a result of caudad growth this pair of excretory ducts extend posteriorly until they open into the cloaca on its lateral wall. Near the nephrostome, but entirely separated from the pronephric tubule, a coiled portion of a small artery covered by peritoneum projects into the cœlom from the body wall. These simple vascular networks, or glomeruli, filter wastes from the blood into the cœlomic fluid. Such wastes, together with cœlomic fluid, are drawn into the pronephric tubules by the ciliary action of the nephrostomes and are then carried down the collecting tubule to the cloaca for expulsion.

A urinary system of this type is the functional kidney of Amphioxus and myxinoid cyclostomes. In other cyclostomes it persists, but yields its place as the functional kidney to the newly formed mesonephros appearing caudad to it. In the embryological development of other higher forms, the pronephric kidney is formed but degenerates, and its functions are taken over temporarily, or permanently, by the mesonephros. The pronephric ducts, however, become the main urinary ducts of the mesonephros.

THE MESONEPHROS.

The mesonephros, or Wolffian body, forms caudad to the site of the pronephros and is composed of more numerous tubules developing metamerically in pairs. These tubules fuse mesially with the pronephric duct, which is now called the mesonephric or Wolffian duct. With development, the mesonephric tubules become gathered into a more or less compact mass, the kidney. In fishes, each

13

kidney is usually an elongated body up under the peritoneum, close to the backbone. In the urodeles the kidneys become somewhat elongated, but in frogs and toads they are shorter and broader. In these latter forms, the kidney becomes free in the body above the viscera but is connected with the body wall by peritoneal sheets of tissue.

The mesonephric tubule is more complex than the preceding pronephric tubule. In the embryonic tubules the proximal end has a distinct nephrostome, as in the case of the pronephric tubule, but differs in that a glomerulus projects into each nephridial tubule in the region near the nephrostome. The arterial tuft is thus surrounded by a double capsule of reflected tubular tissue, Bowman's capsule, and the whole is called a renal corpuscle. In such an arrangement the fluid from the blood is filtered through the inner capsule and passes directly into the lumen of the mesonephric tubule without first passing into the cœlomic fluid, as in the case of the pronephros. With development the nephrostomes may close; in urodeles they persist through life but many of them degenerate in anuran kidneys. Continuing from the glomerulus there is a proximal secreting portion of tubule that in turn connects with the mesonephric duct.

The mesonephros is the functional kidney of most adult cyclostomes, elasmobranchs, teleosts, and Amphibia. It is functional for a short time in certain young lizards and for a short time after birth in the monotreme, Echidna, and in the marsupial, Didelphys. The kidney of the frog may be taken as an example of the functional mesonephros.

Kidney of the Frog.—The kidney of the frog is a flattened, relatively broad, elongated body lying within the body cavity dorsal to the viscera. It is composed of an aggregate of mesonephric tubules whose distal ends unite with the main collecting duct, the mesonephric duct, which runs along the outer lateral edge and continues posteriorly to join the cloaca.

Each tubule has several different regions. At the proximal end of each is the renal corpuscle (Fig. 123), formed by a glomerulus of arterial capillaries encapsulated by the double layer of thin, flat, epithelial cells forming Bowman's capsule. As the tubule continues distally the cells become cuboidal, or low columnar, and are more distinctly outlined. The region of the tubule just distal to the capsule is formed by ciliated cuboidal cells and may receive the short branch composed of ciliated cuboidal cells that opens to

the nephrostome. After leaving the region of the renal corpuscle, the tubule continues as a larger proximal convoluted portion. This convoluted portion formed by low columnar cells with a brush border then joins a short constricted region of the tubule composed of ciliated cuboidal cells. Passing from the constricted portion, the tubule again becomes wider and convoluted, forming the distal convoluted portion which is composed of cuboidal cells often showing cytoplasmic striations, and fuses with a short, straight, connecting piece that empties with others of the same kind into a larger collecting duct. A number of these collecting ducts join the mesonephric duct which runs along the outer edge of the kidney.
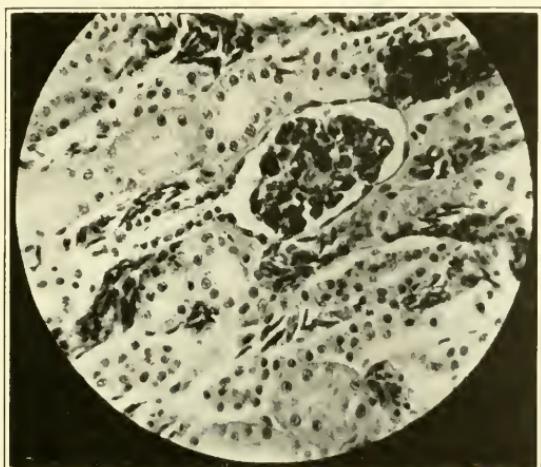
Fig. 123.—Photograph of a section of the bullfrog's kidney (Mesonephros), showing a glomerulus surrounded by Bowman's capsule which continues into the neck of the uriniferous tubule. Sections of nephridial tubules and adjoining capillaries surround the renal corpuscle.

These different portions of the tubules are distributed in a more or less definite manner in the kidney. In the ventral region are located the distal convoluted portions; in the mid-region the renal corpuscles and constricted portions; and in the dorsal region the proximal convoluted portions and the collecting branches. The adrenal gland appears as a wavy golden line located along the mid-ventral surface. In the male, the vasa efferentia from the testis unite with the main collecting duct along the lateral edge of the kidney. The Wolffian duct, therefore, serves as a sperm duct during the breeding season. On the ventral surface adjacent to the renal corpuscles the nephrostomes are to be found when present.

Amphibian kidneys have a peculiar blood supply. Branches of the dorsal artery, called renal arteries, carry arterial blood to them, and venous branches from the kidneys unite to form the postcaval veins which return blood to the heart. In addition to these, renal-portal veins arising from veins in the legs also pass to the kidneys. The renal arteries are connected with the glomeruli of the renal corpuscles. The capillary network around the tubules is derived from the renal-portal veins. All the blood returns to the heart *via* the postcava.

Despite many physiological experiments with the frog's kidney, the manner of its functioning is not thoroughly understood. Observations of the living kidney have shown an intermittent flow of blood through the glomeruli, with some glomeruli thus being retired from or recalled to active functioning as conditions demand. The neck region and the narrow ciliated portion at the lower end of the proximal convoluted tubule have been observed to contract, and presumably regulate urinary flow. The flow of urine from the distal convoluted tubule to the collecting duct is rapid. Experiments indicate that the glomerulus filters urine from the blood, that the proximal convoluted tubules concentrate this filtrate by resorbing water to a greater extent than other substances, and that the ciliated portions before and behind this region act as valves and, together with the distal convoluted tubules, constitute the pressure regulating portion of the kidney.

THE METANEPHROS.

The metanephros develops from the caudad nephrogenic tissue near the cloaca at the base of the mesonephric duct, and is composed of a compact mass of tubules. Like the mesonephros, which precedes it in time and location, this kidney has a double origin. The Bowman's capsule and secreting portions of the tubules are formed from the caudad nephrogenic tissue, but the collecting portions arise as outgrowths of the mesonephric duct, which was formed at the time of the pronephros appearance and retained after the degeneration of the tubules of that kidney. The glandular portion of the entire tubule, *i. e.*, that part developing from nephrogenic tissue, is called a nephron. At about the time when nephrogenic tissue begins developing metanephric tubules, a bud evaginates from the bend of the mesonephric duct where it joins the cloaca. This bud pushes forward to the anterior lumbar region of the body cavity,

where it expands and becomes associated with the nephrogenic tissue. The duct portion of this prolonged evagination becomes the ureter, or main excretory duct of the metanephros. The expanded anterior portion is the primitive pelvis and gives off anterior buds that form the calyces from which branching evaginations give rise to systems of branched collecting tubules. The latter fuse with the nephridial tubules that have been developing during this same period. This collecting system forms a conical mass of tree-like branched collecting tubules that constitute the major portion of the medulla of the kidney and extend radially into the cortical portion occupied mainly by the secretory tubules with which they fuse.

The nephridial tubules of the metanephros are more complex and more numerous than those of the mesonephros and do not at any time have a nephrostome associated with them. Bowman's capsule closely surrounds the glomerulus and continues distally to the neck of the tubule. Continuing from the neck, the tubule becomes convoluted for some distance, then constricts and straightens to descend and loop back to form a wider distal convoluted portion. The distal convoluted portion finally joins with a collecting tubule by a junctional tubule or in some cases by a short arched portion.

This type is the permanent kidney of reptiles, birds, and mammals, but before its appearance the other two types successively precede it in embryological development. The caudad portion of the pronephric duct remains as the mesonephric duct, and this in turn by evagination from its caudad portion gives rise to the ureter of the metanephros, and the original main portion of it becomes converted into the male genital duct, the vas deferens, for these forms.

As an example of this type the mammalian kidney may be used, since details of its structure are well known and give all the structural characters.

Mammalian Kidney.—Mammalian kidneys are bean-shaped organs located in the lumbar region against the dorsal body wall. (Fig. 124.) They appear to be free in the body cavity, but are held in place by the sturdy trunks of the renal arteries, renal veins, and by the ureters. The peritoneum, which splits into connective-tissue sheets or fascia at the outside border of each kidney, also aids in support. The posterior fascia unites with the front of the spinal column, and the anterior fascia covers the front of the kidney and its vessels, passing over the aorta to meet the corresponding fascia from the other kidney. There is usually considerable fatty tissue

between the kidney and its fascia. The kidney of the cat is about 3 cm. long by 2.5 cm. wide and 2 cm. thick; the human kidney is about 11 cm. long, 6 cm. wide, and 2.5 cm. thick, with the long axis parallel to the backbone. The outer margin of each kidney is convex, but the mesial border presents an indentation, called the hilus or hilum. Here the renal artery, renal vein, and ureter connect with the kidney. The entire kidney is covered with a loose capsule of connective tissue which is similar to the peritoneum in composition. There is little connective tissue within the substance of the kidneys, although the capillaries are accompanied by a rich reticular network.
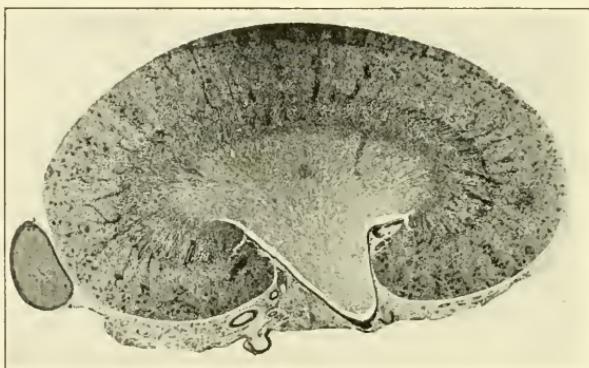
Fig. 124.—Photograph of a longitudinal section of a rat's kidney, showing a single papilla extending into the calyx. The darker striated region surrounding the papilla (medulla) is the cortex. The adrenal is shown closely attached to the kidney on the left.

If the entire kidney is divided lengthwise into two halves by a plane parallel with the adjacent body wall, additional features can be seen macroscopically. At the hilus can be seen the expanded funnel-like continuation of the ureter, called the pelvis, the internal end of which is further subdivided into small funnel-like divisions, called the major calyces, each of which divides into minor calyces. The main collecting tubules open into the minor calyces. (Fig. 125.)

The body of the gland is divided into two zones, an external zone just within the capsule, called the cortex, and an inner zone, the medulla, toward the pelvis. Toward the hilus of the human kidney the medulla is divided into a number of triangular masses, with the base of each directed toward the cortex and the apex of each fitting into a minor calyx of the pelvis. These triangular appearing masses are known as renal pyramids and are in reality conical masses of

tubules. The human kidney may have twenty renal pyramids, but usually they are not so numerous. The kidneys of marsupials, insectivors, rodents, and carnivors have usually only a single renal pyramid. In the medullary zone between the renal pyramids are branches of the renal artery and vein embedded in connective tissue; these interpyramidal regions are known as the columns of Bertini. With the aid of a hand lens or low-power objective, an examination of the cortex reveals alternating light and dark radial striations. The lighter striations are called pars radiata. The darker striations are called pars convoluta and show a series of small dot-like struc-
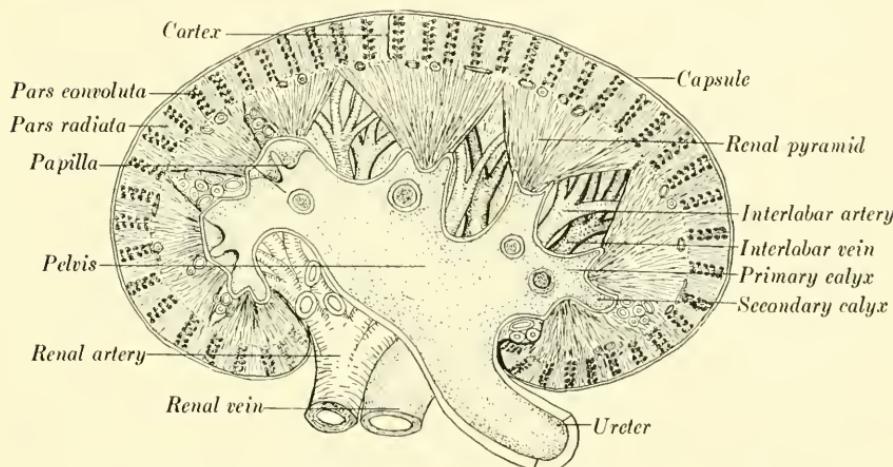
FIG. 125.—Diagram of a longitudinal section of a human kidney.

tures, renal corpuscles, arranged along an imaginary radial axis. Before progressing further it is essential to understand in detail the structure and distribution of a nephridial tubule which is the microanatomical structural unit of the kidney. Each kidney possesses thousands of these nephridial tubules.

The Nephridial Tubule.—Attention has already been called to the dot-like structures, the renal or Malpighian corpuscles, visible in the pars convoluta striations. Each nephridial tubule begins with one of these structures, as in the case of the mesonephric tubule. Each renal corpuscle consists of a capillary complex, known as a glomerulus, which is surrounded by Bowman's capsule, a double-walled structure of squamous epithelium. An afferent arteriole carries blood into the glomerulus and an efferent arteriole carries blood away, the two arterioles being adjacent where they continue

into the capillary tuft. The capillary tuft is itself closely invested
with the visceral wall of Bowman's capsule, which fits in close around
the arterioles as they join the capillaries of the glomerulus. This
same inner, or visceral wall, of Bowman's capsule is reflected back
as the outer, or parietal, wall. The parietal wall continues down
around the inner wall and forms a narrow neck at the end of the
renal corpuscle opposite the entrance of the arterioles. The neck
continues into a portion of the nephridial tubule known as the
proximal convoluted tubule. The wall of the neck portion consists
first of low cuboidal and further on of cuboidal and columnar
epithelial cells. The proximal convoluted portion is very much
contorted, extending somewhat toward the surface at first, then
turning toward the medulla and finally continuing into the medulla
as a very much narrower portion, known as the descending limb.
This portion extends radially in the medulla for some distance, then
forms a loop, known as Henle's loop, and turns back as the ascending
limb running alongside the descending limb. The ascending limb
is somewhat thicker than the descending limb. As it passes into
the cortex, the tubule becomes the much thicker and contorted distal
convoluted tubule situated near the proximal convoluted portion.
Toward the cortical surface it joins a collecting tubule by a short
junctional tubule. These collecting tubules join the others which in
turn join larger tubules, until the largest collecting ducts (papillary
ducts) are found in the papillæ of the renal pyramids. These
papillary ducts then join the calyces. (Fig. 126.)

Let us return for a moment to the cortex. A pars convoluta con-
sists chiefly of renal corpuscles, together with proximal and distal
convoluted portions of the nephridial tubules. A pars radiata is
formed mainly by collecting tubules. The medulla consists chiefly
of collecting tubules, together with the descending and ascending
limbs, and Henle's loop. Little has been said about the blood-
vessels involved in all these regions, for the vascular supply will be
considered separately later.

As indicated, a number of branchings, about seven, occur between
a given papillary duct and the smallest collecting tubules draining
into it. Each papillary duct is like a tree with many branches, the
collecting tubules of different grades originating from the single
trunk. All of these branches constitute parts of the collecting sys-
tem and have the same origin as the calyces, pelvis, and ureter in
the evagination of the mesonephric duct. The nephridial tubule
proper, which develops from nephrogenic tissue and takes part in

the elaboration of urine, consists of those parts from Bowman's capsule through the distal convoluted portion and may be called the nephron. The main function of the excretory tubule is to conduct urine from the secretory tubule, or nephron, to the pelvis. (Fig. 126.)

Connecting tubule

Distal convoluted tubule

Renal corpuscle

Proximal convoluted tubule

Descending limb

Ascending limb

Collecting duct

Loop

Papilla

Capsule

Afferent arteriole

Efferent arteriole

Renal corpuscle

Interlobular vein

Interlobular artery

Arched artery

Arched vein

Interlobar vein

Interlobar artery
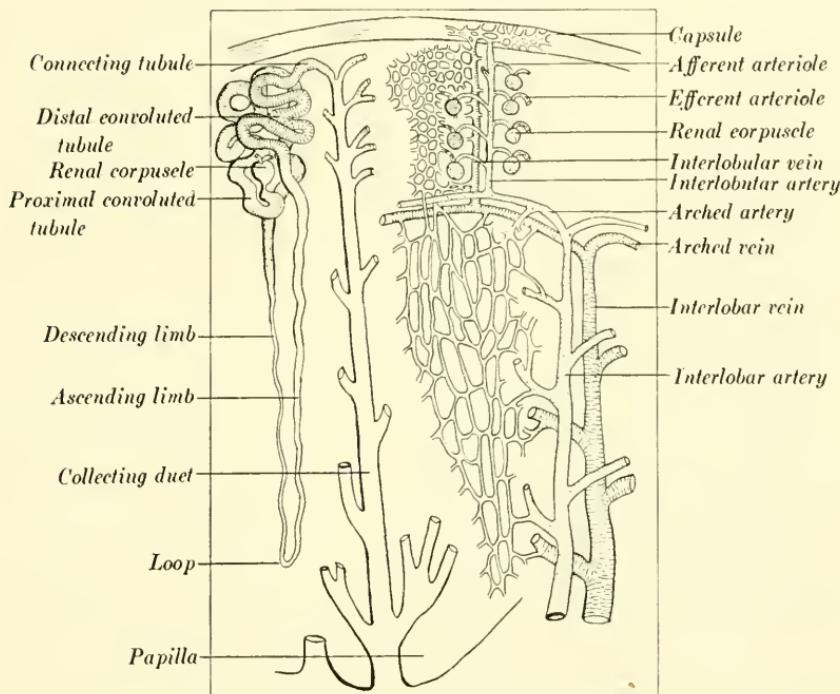
Fig. 126.— Diagram of the uriniferous and vascular system of a mammalian kidney.

Blood Supply of the Kidney.—The renal artery and renal vein divide into trunks, of which the greater number pass over the front of the pelvis in man but ventrally in quadruped mammals. Branches of these, known as interlobar arteries and veins (columns of Bertini) extend between adjacent pyramids to the boundary between cortex and medulla. There, as arcuate arteries and veins, they curve at right angles to lie between the cortex and medulla. From the arcuate arteries, small vessels (interlobular arteries) extend radially toward the cortex. From each interlobular artery a number of afferent glomerular arterioles originate, one to each renal corpuscle. Each efferent glomerular arteriole forms a capillary network in the cortex surrounding the convoluted tubule of that

nephron, and extends alongside the collecting tubules of the cortex. This capillary network connects with the interlobular veins running alongside the interlobular arteries, and then these join the arcuate veins. The capillary system of the cortex joins with the capillary supply of the medulla. Here the capillary network surrounds the systems of collecting tubules and those portions of the nephridial tubules (nephrons) which are located in the medulla. The capillary network of the medulla connects with radially directed venulæ rectæ, which are small veins connecting with the arciform veins. The latter branches join to form interlobar veins, which occur alongside interlobar arteries and eventually join with the renal vein. Not only is the kidney well supplied with arteries, veins, and capillaries, but there is also a rich lymphatic system. The nerves supplying the kidney are branches of the sympathetic plexus.

Fig. 127.—Photograph of a cross-section of the ureter of Necturus in the region of the kidney.

THE URETERS.

Urine is conveyed from kidneys by tubes called ureters. The ureter of a pronephros is a pronephric duct and posteriorly this duct joins with the cloaca. The posterior portion of this same duct also serves as the ureter for the mesonephros, in which case it is known as the mesonephric, or Wolffian, duct. As an example of the structural character of this duct in the case of the Amphibia, the frog's ureter will serve.

Mesonephric Duct.—In the frog, the ureter runs dorsally along the anterior two-thirds of the kidney, then turns to accompany the portal vein along the outer border. During its course along the kidney, the ureter is inclosed in the fibrous connective tissue of the kidney. In the remainder of its course, it lies free in the body cavity associated with the Müllerian duct. The mucous membrane of the ureter is longitudinally folded and varies from 1 to 3 cell layers in thickness. (Fig. 127.) In the anterior portion along the kidney, there may be a single layer of cuboidal or low columnar

cells, but the mid-region along the kidney has a superficial layer
of columnar cells and a basal layer of polyhedral cells. Through the
main portion of the ureter from the kidney posteriorly the superficial
cells, which may have smaller cells interspersed, rest upon one or
two layers of polyhedral cells. Immediately below the epithelium
is a thin region of connective tissue surrounded by a circular band
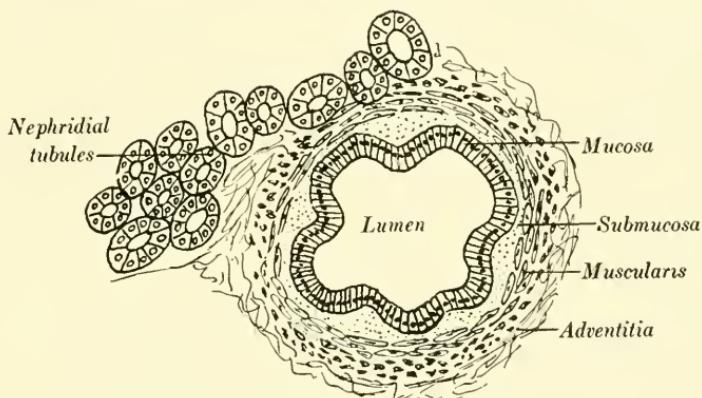
FIG. 128.—Diagram of ureter of the water snake and adjoining uriniferous tubules.
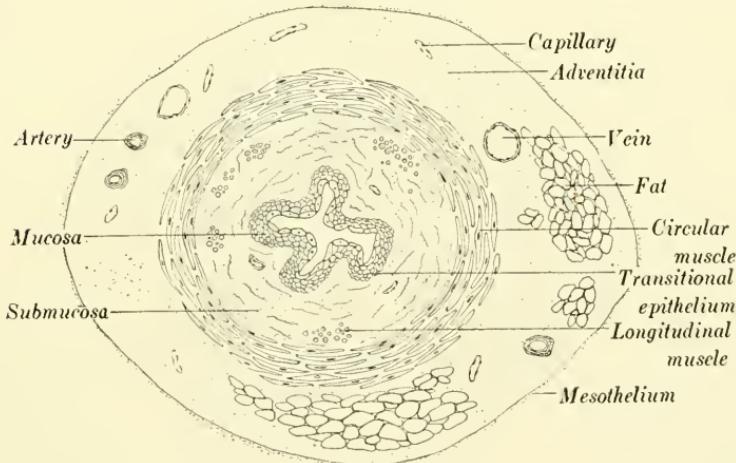
FIG. 129.—Diagram of mammalian ureter (dog).

of smooth muscle. A thin layer of connective tissue forms the most
external coat.

Metanephric Duct.—In reptiles, birds, and mammals where the
metanephros is the functional kidney, the ureter occurs as a new
structure developing from the basal portion of the old mesonephric
duct, as already noted. (Fig. 128.)

In the case of mammals, the ureter has three distinct coats. The lining, like that of the kidney pelvis with which it is continuous, is composed of transitional epithelium. This with an underlying tunica propria of loose fibroelastic connective tissue constitutes the mucosa. (Fig. 129.) It has longitudinal folds, so that in cross-section the lumen appears to be star-shaped. Outside the mucosa there is a muscularis coat which usually exhibits an inner layer of scattered longitudinally arranged strands of smooth muscle cells and an outer, thicker zone of circularly disposed smooth muscle cells. These two sets of muscle cells produce the peristaltic motion which continuously carries drops of urine away from the kidney. Covering the muscularis coat is the fibrosa which is composed of fibroelastic tissue and connects the ureter with adjacent structures.

THE BLADDER.

In most vertebrates there is some provision made for the temporary storage of the urine which is continuously being excreted from the kidney. The urine is collected in sacs, called bladders, of which there are three types, namely, the tubal, the cloacal, and the specialized bladder of the Amniotes.

The tubal bladder is found in many fishes where the posterior ends of the ureters (Wolffian ducts in this case) are enlarged for temporary urine storage. In some fishes there is a partial fusion of the two expanded ends, and in others there is a fusion of the two into a single large sac with which the ureters are connected. In the latter case there is a single exit located in a little eminence, called the urogenital papilla, which is in the wall of the cloaca and into which the genital ducts also open.

A *simple cloacal bladder* is found in the lung fishes and amphibians. It is a thin-walled sac formed as an evagination from the ventral wall of the cloaca. In the frog, it is lined with a pseudostratified epithelium. A thin layer of connective tissue containing irregularly arranged smooth muscle cells is subjacent to the epithelial covering. An outermost region of connective tissue similar to the peritoneum completes the structure of its wall. Such a bladder is anchored to the body wall by a dorsal mesentery of peritoneum and similar attachments from the lateral faces to the body wall. This type of bladder stores urine which collects in it from the closed cloaca. Stored urine is ejected by contractions of the body wall. The allantois of the amniotes also arises as an evagination of the cloacal wall and is homologous with the amphibian cloacal bladder.

The *bladder* occurring among the amniotes has been called an allantoic bladder, but embryological studies have shown that the allantois is not really concerned with the formation of the bladder of these forms. Ophidia, Crocodilia, many Lacertilia, and Aves have no bladder. Turtles, some lizards, and all mammals have one. The bladder of the monotremes is said to be homologous with that of amphibians.
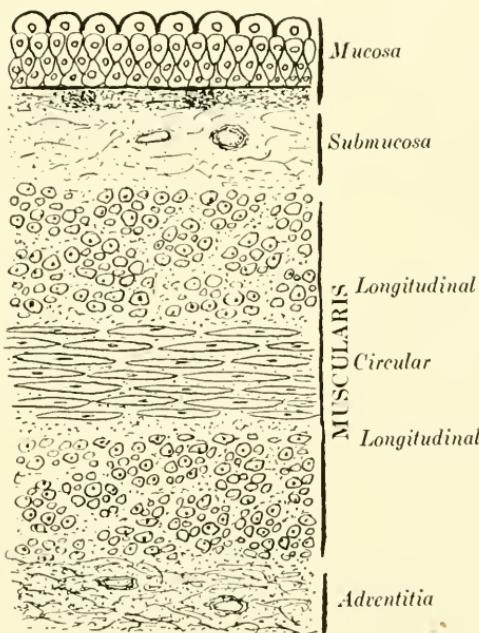
Fig. 130.—Diagram of mammalian bladder.

An idea of the development of the mammalian bladder may be gained from a study of its development in man. In embryos, the posterior end of the embryonic gut is somewhat expanded and ventrally lies against the ectodermal body wall. Later in this region an endodermal diverticulum from the cloaca will meet an ectodermal invagination to form the anal opening. From the bend where each mesonephric duct joins the cloaca a bud will form, and from this will develop the ureter of the metanephric kidneys already described. Somewhat later the cloaca in this region subdivides into two passages entirely separated from each other. The more dorsal of the two forms the rectum, and the ventral forms a urogenital sinus. This occurs when the embryo is about eight weeks old in humans,

and while it is taking place the urogenital sinus itself is subdividing into a dorsal and ventral portion. The allantois and mesonephric ducts connect with the dorsal portion of the urogenital sinus. The ventral, so-called phallic portion, is later involved in the development of the urethra region of the genital ducts of both sexes, especially with the penis of the male, and continues to expand to become a wide, muscular-walled sac, the bladder. At the anterior ventral end of this enlargement is a stalk of tissue, the urachus of the embryo, but in the adult this is the middle umbilical ligament tying the bladder to the body wall. The ureters shift their position so as to join the wall of the developing bladder, and as the latter grows forward it carries the ureters with it, separating them from the mesonephric ducts which are retained to become functional in connection with the reproductive system of the male.
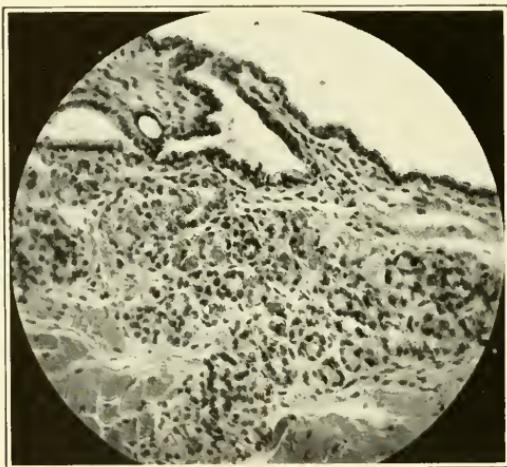
Fig. 131.—Photograph of the urethra of a female mouse. The lumen is lined by a thin stratified epithelium. In the submucosa are mixed serous and mucous glands which extend into the underlying skeletal muscle.

The mammalian bladder has four coats of tissue making up its wall, namely, the mucosa, submucosa, muscularis, and fibrosa. (Fig. 130.) The mucosa, like the ureters, is lined with transitional epithelium. The behavior of this type of epithelium in the distended and empty bladder has already been described in the consideration of epithelia. In the empty bladder the mucosa is wrinkled by folding of the underlying submucosa. In some bladders there are simple gland-like pockets in the epithelial wall. There being

no muscularis mucosa, the submucosa of fibroelastic connective tissue unites with the tunica propria underlying the epithelial lining. A study of a cross-section of relaxed bladder wall shows a thick muscularis. The muscularis coat is divided into a thick middle coat with circularly arranged cells, an innermost region with a thin layer of longitudinally arranged cells, and an outside region also with longitudinal layers of cells. The muscle tissue, although loosely arranged, is very strong. The fibrosa consists of fibroelastic connective tissue and merges with a peritoneal-like coating. At the junction of the bladder with the urethra there is a ring of circularly disposed smooth muscle, the internal sphincter of the bladder.

THE URETHRA.

The urethra differs in the male and female. In the male it forms the duct of the penis, and an account of its structure will be considered with the male system of reproduction. The female urethra is short. (Fig. 131.) It extends from the bladder to the vestibule and opens into the vestibule along with the vaginal part of the female reproductive system. Internally it presents longitudinal folding of the mucosa. The epithelium of the mucosa is commonly of the stratified squamous variety. There is no distinct submucosa; outside the tunica propria is a muscularis coat with an inner longitudinal coat and an outer circular coat. Outside the muscularis is a fibrosa. Near the junction of the ureter with the vestibule there is a sphincter muscle of the skeletal variety outside the smooth muscle.

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See Appendix also.

THE FEMALE REPRODUCTIVE SYSTEM.

AMONG the vertebrates, the sexual systems are generally separated, different individuals developing into either male or female, but for a time during the early embryology the male and female sexual systems follow the same developmental plan and are indistinguishable either anatomically or histologically. Elements of both systems are developed at this time, though genetically sex is determined at the time of fertilization. In early vertebrate embryos the urogenital fold develops on either side of the dorsal mid-line. From the greater part of this fold the urinary system develops, as already described; but from the mesial ventral surface a longitudinal thickening arises to form the genital ridge. This ridge is covered with a cuboidal epithelium and separated from the developing kidney by mesenchyme. The simple epithelium of the genital ridge proliferates into a stratified zone of polyhedral cells in which two types of cells soon make their appearance. Larger spherical cells, the primordial sex cells, are scattered among the cuboidal or polyhedral indifferent cells, and the underlying mesenchyme forms string-like masses projecting into the epithelial mass. This mass represents the early indifferent stage in development of the sex glands, or gonads. The excretory ducts of the genital system are also closely associated with the urinary system. Two longitudinal ducts are developed on each side from the mesoderm lining the cœlome, and these open caudally into the cloaca. The one duct is the Wolffian duct, already described in considering the excretory system, the other is the Müllerian duct, which becomes the oviduct of the female. With development, sexual differentiation occurs, and the system associated with the sex so determined progressively differentiates while the elements of the other system degenerate.

THE OVARIES.

The female organs of reproduction consist of the ovaries, in which the ova are produced, and a pair of ducts, the oviducts, by which the ova are conducted away. The ovaries of vertebrates are usually paired organs, but occasionally, as in elasmobranchs and birds, one

14

ovary degenerates, or, as in certain teleosts, the two ovaries fuse.
In the embryonic gonad, string-like continuations of the underlying
mesenchyme divide the epithelial cells into cords and carry along
developing blood vessels. Thus, each gland is composed of spherical
cells supported by connective tissue, and the whole covered by an
epithelial membrane continuous with the peritoneal lining of the
cœlome. The first indication of ovarian differentiation appears in
the development of a layer of the cuboidal or polyhedral cells to
form follicle or nurse cells about larger primordial sex cells. Gen-
erally a single layer of cuboidal or columnar cells surrounds each
maturing ovum and assists in the elaboration of the nutrition for
its development. By the activity of these cells, yolk is added in
the case of yolk-bearing eggs so that adequate nutrition is stored
for future developmental demands to be met after fertilization.

Not all the ova and their follicle cells complete their development.
A number appear to reach various stages of differentiation and then
disintegrate and are resorbed with an accompanying invasion of the
surrounding connective tissue.

The condition of the ovary shows considerable variation in the
different groups of vertebrates and the development of the ovum
likewise varies. Among the oviparous forms the fertilized egg
develops outside the body and must depend upon its stored nutri-
tion. In some viviparous forms it may likewise have to depend
entirely or in part upon stored nutrition. In placental mammals,
no yolk storage occurs and nutrition is furnished the embryo directly
from the mother throughout its development.

Fishes. — In myxinoid cyclostomes, an unpaired, elongate gonad
is held by a median dorsal mesentery. No genital ducts are present,
and the ova and sperm are liberated into the body cavity from which
they pass to the urogenital sinus by two pores. The anterior
portion of this gonad produces ova and the posterior portion pro-
duces sperm, but both are not functional at the same time. In
this case an individual functions first as a male and then as a female.
In some cyclostomes only one portion may be functional, though
the other is present.

In elasmobranchs, such as the skate, the female has a single
ovary; the right ovary degenerates. In the functional left ovary,
ova periodically mature in round follicles on the periphery and are
liberated to be picked up by a pair of oviducts opening behind the
pericardium. The eggs of this group are the largest among the
fishes and usually only a small number mature each season.

The teleost fishes produce the smallest eggs generally and also the most numerous; sometimes millions may be formed each season. The ovary here arises as an evagination of the embryonic wall of the body cavity on each side of the mid-line, and becomes surrounded by a fold of the peritoneum which encloses it like a sac and continues caudally to form a duct connecting the ovary with the cloaca. In this case the eggs do not pass into the general body cavity when mature, but continue down this duct to the cloaca.

In general, the ovaries vary from an inactive stage, in which few oögenic cells occur in the connective tissue of an elongate membranous fold, to the active stage, in which the ovary becomes filled with developing ova. In the active stage the connective-tissue covering of the ovary is extremely thin, and the mass of maturing ova are visible through it. The oögenic epithelial cells of the ovary become active before each breeding season, and oöcytes and follicle cells are proliferated. Each ovum becomes surrounded by a layer of flattened nurse or follicle cells and a delicate sheath, or theca, of connective tissue supporting a capillary network. As the ova mature, a storage of yolk occurs in the cytoplasm and they distend the connective tissue until, at the time of ovulation, they break through the follicular sheath and ovary wall into the body cavity. Cells derived from the follicle fill in the cavity left by the ovum and form a corpus luteum.

Amphibians.— Nearly all of this group are oviparous and lay their eggs in water. During the breeding season, when one opens a female amphibian, such as a frog, a mass of eggs is found occupying the greater portion of the abdominal cavity. These are not free in the body cavity, but are held in a pair of swollen sacculated ovaries covered by a reflected fold of peritoneum. Dorsally this peritoneal fold extends from each ovary to attach the ovary to the dorsal wall of the body cavity and is continuous with the peritoneum. This fold, the mesovarium, carries blood vessels, nerves, and lymphatics to each ovary. Beginning in the region of the mesovarium, the oögenic cells of each sacculated portion of the ovary begin proliferating for the reproductive season. Each maturing ovum becomes surrounded by a layer of cuboidal cells that gradually elaborate more and more yolk which is added to the cytoplasm of the ovum. Surrounding this layer of nurse cells is a delicate sheath, or theca, of connective tissue. Continued proliferation and consequent maturation of the eggs distends the ovary and its peritoneal covering to the point observed above. When mature, the eggs

break from their surrounding follicle and through the saccular
covering of the ovary into the body cavity from which they pass
into the oviducts. After the mature eggs of the breeding season
have been discharged, the ovary collapses to a small body having
the appearance of a bit of folded whitish membrane. Within this
membrane are the small undeveloped oögenic cells that will give
rise to ova of the succeeding breeding seasons. (Fig. 132.) The
gelatinous mass that surrounds the discharged eggs of amphibians
is added by the oviducts as the eggs pass outward.
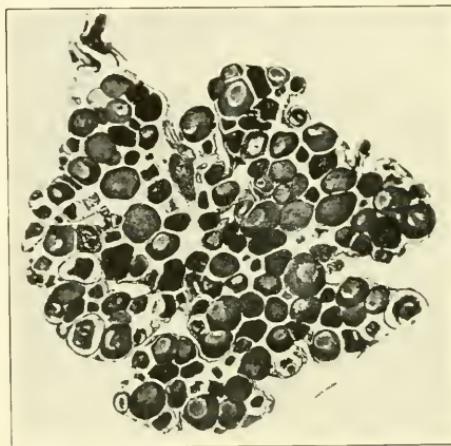
FIG. 132.—Photograph of a section of an ovary of the frog. The ovarian tissue
is composed of several sacs, each containing a number of immature ova supported
in a delicate connective tissue.

Reptiles.—The ovary of a reptile during breeding season is com-
posed of a number of eggs of various sizes. In the lizard, for example,
the ovaries are not sacculated, as in the case of the frog, and the
germinal center is quite compact and more easily discovered.
(Fig. 133.) In it are developing oöcytes and a series of develop-
mental stages continuing into an adjacent string of ova of increasing
size. At first each ovum is surrounded by a single layer of nurse
cells, but these cells become more numerous and stratified as the
egg increases in size. The increase in egg size is due to the increas-
ing amount of yolk supplied to the ovum by the surrounding nurse
cells. The size of the eggs, therefore, indicates the relative age, the
largest being the most mature, the smallest the least mature. A
theca of vascular connective tissue surrounds the follicle cells.
When the elaboration of yolk is completed, a rupture occurs in

the connective-tissue theca of the follicle and the connective-tissue
covering of the ovary. The egg is discharged from its follicle into
the body cavity, where it is picked up by an oviduct, fertilized
by sperm present, and carried outward. In some cases develop-
ment is internal, but more commonly it is external.

Mammals.—The ovaries are small, round, or oval bodies, one
located on either side of the mid-line near the dorsal wall just
posterior to the kidneys. Along the mesial border there is an inden-
tation, or hilum, where the mesovarium connects the covering tissue

Fig. 133.—Photograph of a section through two oögenic masses in the ovary of a
lizard. A series of maturing ova is shown on the left and another series on the right.
Even the largest eggs are immature and still retained within the connective-tissue
sheath.

of the ovary with the peritoneum of the body wall. The meso-
varium is surfaced with mesothelium similar to that of the perito-
neum and continuous with the cuboidal or low columnar epithelium
covering the ovary. The epithelium covering the ovary is spoken
of as the germinal epithelium, and within it the ovary is divided
roughly into two zones. An outer portion immediately beneath
the germinal epithelium is the cortex; an inner region below
the cortex and toward the hilum is the medulla. The connective
tissue of the cortex is of the fibroelastic variety, with an additional
network of reticular tissue. There are also present spindle-shaped
cells, capillaries, and small blood vessels. Just beneath the germinal
epithelium there is usually a dense region of encircling connective-

tissue fibers forming the region known as the tunica albuginea.
Distributed through the cortical connective tissue are the primary
ovarian follicles composed of maturing ovum surrounded by a layer
of follicle cells.   In the medulla, ovarian follicles are absent; there
are many elastic fibers, scattered smooth muscles, and the larger
branches of the ovarian vessels in a fibroelastic tissue stroma.
(Fig. 134.)
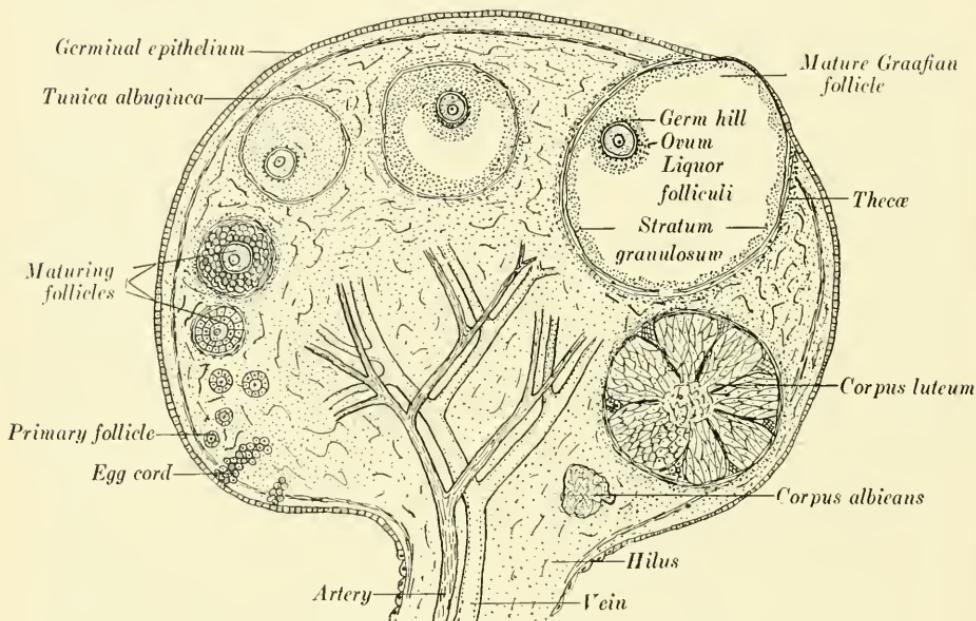
FIG. 134.—Diagram of a mammalian ovary.

Groups of special cells derived from a particular portion of the
connective tissue have been identified in some ovaries as interstitial
cells believed by some to have an endocrine function similar to the
interstitial tissue of the testes.   These cells, when present, are large,
polyhedral, epithelioid cells with lipoid droplets in their cytoplasm.
There is no evidence to confirm the conjecture that they are endo-
crine or that they act as intermediaries for nutritive transfer between
the blood vessels and the ovarian follicles.

The conditions of the ova and follicles in the cortical region of
the ovary vary with the animal under consideration and with the
age of each individual.   Beginning with an indifferent gonad in
which primordial sex cells are scattered among the indifferent cells,
there is a progressive differentiation.   The cuboidal epithelium

covering the embryonic ovary proliferates cords of cells, called
Pflüger's egg cords, which extend in toward the embryonic connec-
tive tissue and earlier formed germinal cells. At first there is no
clear differentiation possible between the cells that will become the
ova and those that will form the follicle cells. Later on, regions
of the cord segment and a large central cell, the oöcyte, is surrounded
by several of the smaller follicle cells. The early ovum surrounded
by the several flattened follicle cells is known as a primary follicle.
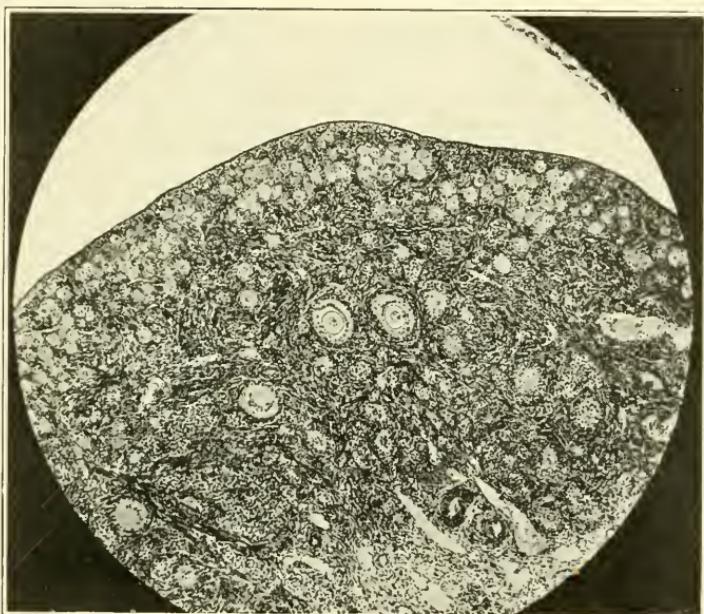
FIG. 135.—Photograph through the cortex of the ovary of a new-born dog. Along
the outer margin is a region with many primary follicles. A few developing follicles
occur in the deeper region toward the medulla.

(Fig. 135.) Just before birth in man, and shortly after birth in some
of the other mammals, the up-growing mesenchyme develops the
tunica albuginea subjacent to the germinal epithelium. The for-
mation of this layer of connective tissue prevents further develop-
ment of cords of cells from the germinal epithelium. During this
period the primordial sex cells and indifferent cells in the deepest
regions of the ovary have degenerated, and this region becomes
the medulla. The cortex now contains all the remaining primary
follicles, and some of these begin a progressive development pre-
liminary to sexual maturity and the beginning of a reproductive

cycle. Thousands of primary follicles are present in the ovaries of a new-born female, but most of these undergo gradual degeneration and resorption, a process known as atresia. The primary follicles that are to mature undergo a complex development before the ovum is ready for fertilization.

Maturing Primary Follicles.—The rudimentary ovum, or oöcyte, is only slightly larger than the surrounding follicle cells. Both the nucleus and cytoplasm of this cell increase in size with an accompanying increase in the number of surrounding cells which are now cuboidal in appearance. During this early period the chromosomes of the oöcyte are organized for the first maturation division, after which the chromatic material returns to the reticulated condition characteristic of these cells. The second maturation division occurs after the egg leaves the ovary. A highly refractive membrane, the zona pellucida, forms between the cytoplasm of the ovum and the surrounding polyhedral cells of the follicle which have multiplied to form several layers, the stratum granulosum. Ensheathing the granulosum there is a thin layer of connective tissue, the theca folliculi, which carries a capillary network. The cells of the stratum granulosum continue to divide by mitosis and their multiplication increases the size of the follicle. Spaces begin to appear in the mass of cells composing the granulosum region and fill with liquid, the liquor folliculi. The follicle cells continue to increase in number; more spaces and more liquid appear; the spaces begin to fuse and finally form one large space. At one or two points a band of granulosum cells, the cumulus oöphorus, or germ hill, supports the ovum and its surrounding radiating cells, the zona radiata. (Fig. 136.) The ovum and zona radiata are thus separated from the peripheral granulosum cells by the increasing amount of liquor folliculi. With this development there is an accompanying development of the connective tissue immediately surrounding the granulosum to form a theca folliculi divisible into two regions. The inner portion, the theca interna, is composed of connective tissue supporting a network of capillaries and also containing peculiar polyhedral cells with lipoid material in their cytoplasm. Outside the theca interna is the theca externa, composed of denser connective tissue carrying small blood vessels. These maturing follicles are called Graafian follicles.

Ovulation.—The maturing follicle soon occupies the entire width of the ovarian cortex, having increased many times the size of the primary follicles. The germ hill with the enclosed ovum may shift

its position toward the surface and the follicle may cause an appreciable bulge in the ovarian surface. It should be remembered that outside the stratum granulosum at this point are the theca interna, theca externa, tunica albuginea, and the germinal epithelium. Due to the great increase in follicular liquid, all these coats are under considerable tension and are relatively thin. There is finally a rupture at this point, and the liquor folliculi pours out, carrying with it the ovum surrounded by the cells of the germ hill. This rupture at time of ovulation may be accompanied by a hemorrhage into the follicle, so that a corpus hæmorrhagicum is formed.
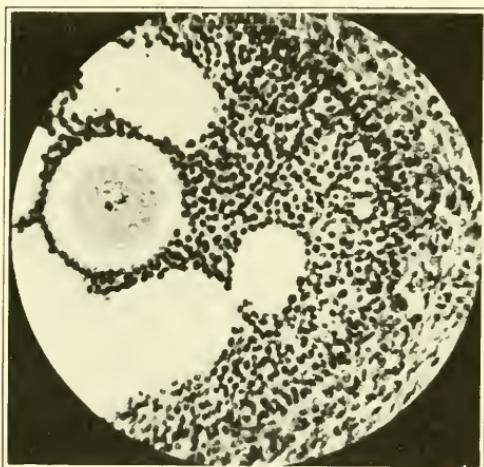
Fig. 136.— Photograph through a follicle in the region of the germ hill (Cumulus oöphorus). Ovary of the dog.

After ovulation, the ovum with the surrounding zona radiata passes into the oviduct, in the upper portion of which the second maturation division occurs and fertilization takes place. There are two sites of continued activity and histological change following normal ovulation, one in the follicular mass and the other in the uterus following the descent of the fertilized egg.

Corpus Luteum. — Following the rupture of the mature Graafian follicle and discharge of the ovum, the stratum granulosum of the follicular wall collapses and folds in so that the previous spherical cavity is reduced and irregular in shape. The cells of the granulosum layer begin to increase in size and number and a yellow lipoid substance forms in their cytoplasm. The yellow color is often prominent and gives the structure the name of corpus luteum at

this period. These cells are now called lutein cells. Strands of reticular tissue migrate radially inward from the theca interna toward the follicular cavity, as do sinusoidal capillaries which extend in between the enlarged granulosum cells. The theca externa remains much as it was before ovulation. If the ovum is fertilized after it enters the oviduct and implants in the uterus, the further changes in the corpus luteum (or follicular body) are quantitatively different than those in the case where no fertilization is effected. In the latter case a corpus luteum spurium is formed, and in the former a corpus luteum verum forms.
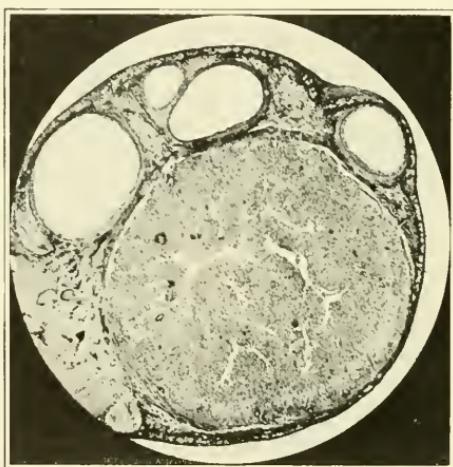
Fig. 137.—Photograph of a section through the ovary of a cat. In the upper region 4 maturing follicles are shown; the light areas were filled with follicular liquid. The large circular body below is a corpus luteum verum. At the lower left margin near this is a corpus albicans.

Corpus Luteum Spurium.—When fertilization does not occur, the corpus luteum reaches the climax of its development and greatest size shortly after ovulation. At this period the yellow color may be pronounced if such color develops at all. Hemorrhage may also occur at this time in the corpus luteum, due to rupture of the capillaries scattered through it, and blood pours into the follicular cavity. This marks the beginning of the degeneration of the corpus, and gradually the whole body is resorbed. As long as the follicular cavity remains slight hemorrhages may occur into it until all that remains is a small mass of connective tissue called the corpus albicans.

Corpus Luteum Verum. (Fig. 137.)—This body is histologically identical with the corpus luteum spurium during the first part of its development. However, if the ovum is fertilized and the embryo implants, the corpus grows larger over a longer period of time than the corpus luteum spurium. Instead of reaching the climax and beginning to degenerate shortly after ovulation it continues to develop and remains present for the greater part of the period of pregnancy. This structure begins to degenerate shortly before birth of the embryo and with birth of the fetus it degenerates rapidly to leave a corpus albicans.

In mammals with relatively short cycles, such as the rat, numerous ova are in the process of maturation and a number mature over short intervals unless fertilization occurs. Numerous corpora are formed in such forms and follicular changes are correspondingly rapid.

THE OVIDUCTS.

There are many variations in the egg-conducting apparatus. In some forms, such as the cyclostomes, and some teleosts, no oviducts are present and the eggs liberated into the body cavity pass to the outside through pores in the cloacal region. In other teleosts, folds of the peritoneum form funnel-like sacs connecting the ovary with the cloaca. They are composed of fibroelastic connective tissue with some smooth muscle and surfaced by a simple epithelial membrane.

In some of the lower vertebrates it appears that the Wolffian and Müllerian ducts arise from a longitudinal splitting of the embryonic pronephric duct. We have already observed that usually the oviducts or Müllerian ducts arise in both the male and female as a fold in the ventral lateral surface of the mesonephric cell mass near its anterior end. The anterior end forms a groove that remains open while posteriorly the fold forms a tube or cord of cells that develops a lumen. The posterior portion grows caudally to fuse with the cloaca or urogenital sinus, but the anterior end remains open into the body cavity and is called the ostium abdominalis. In elasmobranchs, amphibians, birds, and monotremes, the eggs pass into the abdominal cavity and from there into the ostium abdominalis of the oviduct to be conducted to the cloaca.

In vertebrates below the mammals, as a rule, the two oviducts open separately into the cloaca. In mammals, a longitudinal septa, the perineum, divides the embryonic cloacal region into a dorsal

rectum and a ventral urogenital vestibule before birth. In these forms the Müllerian ducts fuse into a single passageway whose anterior portion forms the vagina, which opens into the vestibule separately from the urethra.

The oviducts show modifications depending upon the type of egg and its future development. In the case of the large, shelled eggs of birds and reptiles, there are specialized portions of the ducts which secrete the additional material. In the placentals, the egg is fertilized in the upper portion of the oviduct and then conducted to a lower portion, the uterus, which is modified for implantation and internal development of the embryo.

In general, the oviducts have ciliated columnar epithelium lining the lumen. Simple tubular glands of varying prominence, depending on the season, extend into the subepithelial fibroelastic connective tissue. Loose fibroelastic connective tissue of the submucosa forms folds projecting the mucosa into the lumen. Below the submucosa there is usually a broad coat of circular smooth muscle separated from a less prominent outer longitudinal coat by a region of vascular fibroelastic connective tissue. In the lower regions of the oviducts, compound tubular glands may form relatively large evaginations of the mucosa, as in the skate, composing the shell glands whose secretions are carried into the lumen by numerous excretory ducts lined with mucous columnar cells.

Amphibia.—Amphibian oviducts consist of a pair of long, contorted tubes, each connected with the wall of the body cavity in the dorso-lateral region by a mesenteric sheet. Anteriorly, each opens by a ciliated funnel-shaped ostium into the body cavity; posteriorly they connect with the dorsal wall of the cloaca near the entrance of the ureters. In immature animals and between breeding seasons the oviducts are small, thin-walled structures, but during breeding season there is a great increase in size and they become more convoluted. Over the greater part of its length each duct is of uniform size, but near the cloaca there is a dilated region. The entire duct has an outer adventitia of connective tissue covered by peritoneum. A thin muscularis of circular smooth muscle cells adjoins the adventitia and is difficult to distinguish where the glandular mucosa forms the greater portion of the wall. The lumen is lined by ciliated columnar cells with goblet cells interspersed. Over the greater length of the duct, the mucosa is composed of long tubular glands. These glands secrete the albumen surrounding the eggs as they pass down the oviducts. At the dilated

portions, the walls are thinner and the glands are absent, and the lumen is lined with columnar cells. Eggs may collect in the dilated portion until a female is clasped by a male, when the eggs are liberated and fertilized externally in the water. The secretion of the oviducts absorbs considerable water, and a characteristic mass of gelatinous material surrounds the eggs shortly after fertilization.

Reptiles and Birds. — In the case of reptiles and birds, the albumen surrounding the ovum is produced from glands in the wall of the anterior part of the oviduct, and the shell which surrounds it is formed by glands in the posterior region. Naturally, in cases of animals with shelled eggs fertilization takes place in the oviduct anterior to the shell glands.
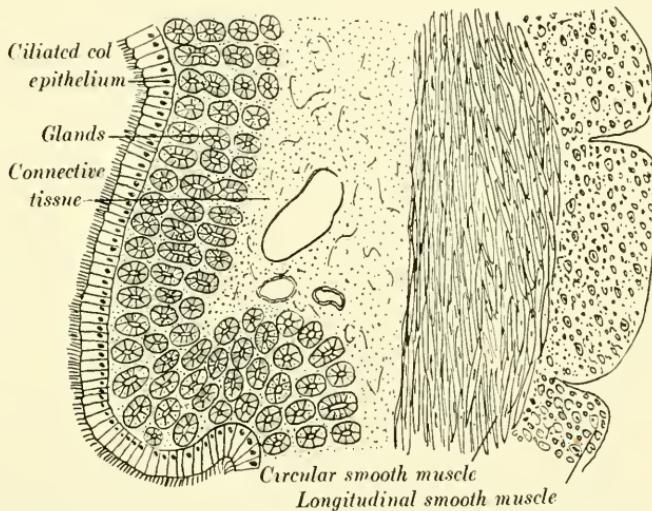
FIG. 138.—Drawing of a section through the oviduct of a turtle.

The wall of the oviducts here is generally composed of the following coats. The mucosa has a lining of ciliated columnar cells and tubular glands that become active and large in breeding seasons. The submucosa of fibroelastic connective tissue forms folds that carry the mucosa into the lumen. The muscularis has a broad circular and a narrower longitudinal layer of smooth muscle. An adventitia, or superficial coat, varies from a minute amount of connective tissue covered by the mesentery to a relatively broad region in some forms. (Fig. 138.)

Mammals. — In monotremes there is still but one external passageway for both the urogenital and digestive systems, the cloaca. In

this instance, however, the male develops a sort of penis, which is inserted for internal copulation. In other mammals there is a urogenital vestibule separated from the anal opening of the digestive tract. Into this vestibule the genital system opens usually by a single passage, the vagina. The upper end of the Müllerian duct functions in conducting the egg to the lower region, or uterus, which is specialized to receive and nourish the developing embryo, and the extreme outer end is modified to receive the copulatory organ of the male for insemination. In some cases the egg-conducting portions, the oviducts or Fallopian tubes, may join a single uterine portion; in others, the lower portion of each tube has a uterine portion and fusion occurs just before the vagina is reached.

In many mammals, including numerous rodents (mice, rabbits, and beavers), certain bats, and other forms, there is a condition known as uterus duplex, in which there are two separate uteri opening into separate vaginæ that fuse near the vestibule. In other mammals, as in certain rodents, some ruminants, and carnivors, there is a condition known as uterus bipartitus, in which there is a partial fusion of the two uteri near their junction with the single vagina. Among certain other ruminants and carnivors there is a greater posterior fusion of the uterus and an anterior prolongation to join each Fallopian tube, a condition known as uterus bicornis. Among the primates generally, there is a complete fusion of the uterine portions to a single uterus continuing into a single vagina, a condition known as uterus simplex.

Each oviduct is a relatively short, narrow, convoluted duct, extending from the ovary toward the mid-line, where it connects with the anterior lateral face of the uterus. The proximal end broadens out funnel-like and tesselated processes surround the ostium abdominalis. These fimbriated processes rather closely cover the ovary. The structure of the tube varies somewhat, but three regions can be distinguished throughout. Externally there is an adventitia of connective tissue surfaced with mesothelium. Medially there is a muscularis composed of an external sheath of longitudinally disposed smooth muscle cells and an internal sheath of circular cells. Internally there is a mucosa of ciliated columnar and occasional glandular cells resting upon a tunica propria of connective tissue. No submucosa is distinguished. Near the ostium the mucosa is elaborately folded, so that sections show a great number of spaces among labyrinthine folds. (Fig. 139.) Toward the uterus these foldings are not so extreme, and the tube wall is

much thicker in proportion to the lumen. The cilia of the columnar epithelial cells beat away from the ostium, so that the egg and follicular fluid are propelled toward the uterus. The epithelial lining may show variations in the appearance of the columnar cells at different periods of the ovarian cycle.

The Uterus.—Regardless of its anatomical variations, whether simplex, bicornis, or duplex, the uterine structure is the same fundamentally. At the end of the oviduct the uterus begins as an abruptly expanded tube containing the same three coats of tissue,
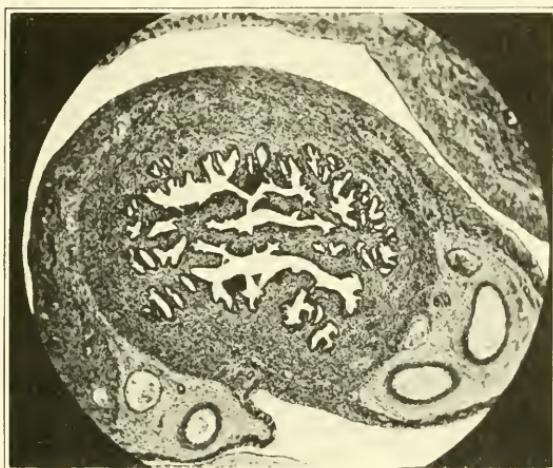
Fig. 139.—Photograph of a section through the oviduct of a kitten near the ovary. The mucosa is much folded.

However, the three divisions of adventitia, muscularis, and mucosa are much more developed than in the preceding portion of the duct. The adventitia is called the perimyometrium, the muscularis becomes the myometrium, and the mucosa is called the endometrium. (Figs. 140 and 141.)

In the connective-tissue framework of the myometrium are many types of connective-tissue cells, including an embryonic variety capable of forming muscle cells in great numbers when pregnancy occurs. In pregnancy the myometrium increases many times its size in the resting uterus, due to increase in cell numbers as well as to increase in cell size. The connective-tissue fibers also increase and there is a looser arrangement and more tissue juice.

The uterus is interpreted usually as having no submucosa; the connective tissue of the mucosa continues into that of the subjacent

muscularis region.   The mucosa or endometrium undergoes remark-
able modifications in cyclical, or seasonal, periods.   (Fig. 141.)   In
humans, a series of changes are repeated each lunar month of the
productive period if pregnancy does not occur.   During each of these
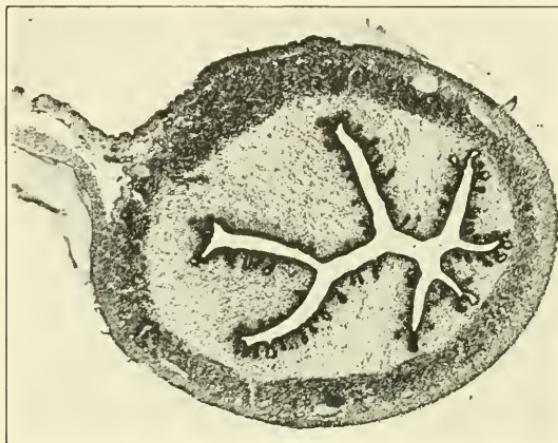
Fig. 140.—Photograph of a section through the resting uterus of the rabbit.   The
endometrium is thrown into several broad folds and many shallow tubular glands
occur in it near the lumen.   The dark band surrounding the endometrium is the
myometrium.   At the left the connective tissue and blood vessels of the mesentery
are shown continuing with the perimyometrium.
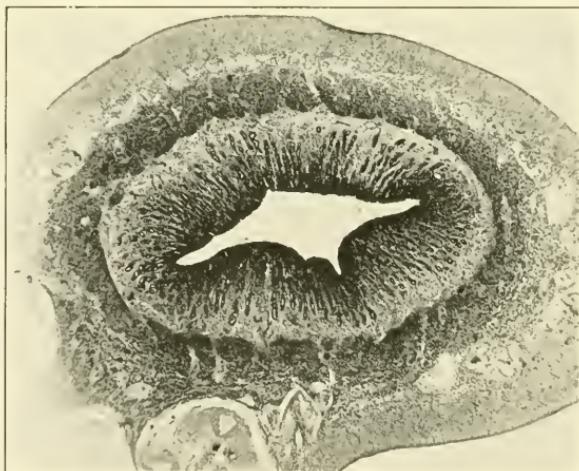
Fig. 141.—Uterus of the cat in heat, showing glandular endometrium and broad
myometrium.

months an ovum is normally discharged from the ovary and, provided
no sperm are present in the oviduct to fertilize the ovum, the special
preparation of the mucosa is not needed. A consequent loss of the
major portion of the mucosa takes place under the name of menstrua-
tion in humans, but in other forms degeneration and resorption
takes place in such cases. Following fertilization, more profound
changes occur in the body of the uterus if the embryo continues to
develop, so a description of the histological structures of the uterus
must also indicate the relation to ovulation and pregnancy. At
certain periods of the lunar month in humans, and at other inter-
cyclic periods for other animals, there is a condition which may be
described as the resting condition of the uterus. The lining of
such a uterus shows longitudinal ridges, due to the folding of the
tunica propria beneath the superficial layer of ciliated columnar
cells. Also in the mucosa are tubular glands extending more or
less perpendicularly toward the myometrium. Some of the colum-
nar cells forming these glands are also ciliated. Just beneath the
superficial epithelium of the lumen and surrounding the gland cells
is a rich reticular network with many mesenchyme-like cells and
leukocytes (especially lymphocytes). In the underlying tunica
propria there is a rich capillary network derived from the vessels
in the myometrium. At its posterior extremity the uterus narrows
to a short neck-like portion, the cervix, which joins the vagina. In
this region the mucosa has only the superficial covering of columnar
epithelium without any glands. The changes that occur in the
uterus preliminary to and following menstruation and pregnancy
will be considered separately under those headings.

The Vagina.—This is a tube-like portion continuous with the
uterus and receives the copulatory organ, the penis, of the male.
The same three coats are present in it as in the anterior portion of
the ducts, an adventitia, a muscularis, and a mucosa. The adven-
titia is similar to that of the uterus and Fallopian tubes. The
muscularis is much thinner than that of the uterus, with an ex-
ternal longitudinal and an internal circular region of muscle tissue.
At the lower end of the vagina the muscularis forms a sphincter
muscle. The mucosa consists of a coat of stratified squamous
epithelium lining the lumen and a subjacent vascular region of
connective tissue, the tunica propria, or corium, which may have
projecting papillæ that throw the epithelium into ridges. Three
zones are distinguishable in the epithelial lining: the one adjacent

15

to the lumen is composed of several layers of flat degenerating cells little, if at all, cornified; the middle zone has several layers of flat cells with a more granular content, suggesting the stratum granulosum of the epidermis; the deepest zone has a number of cell layers comparable to the stratum germanitivum of the epidermis. The basal layer of cells are short columnar and polyhedral in shape and are active in giving rise to new cells. Between adjacent cells of the upper layers of the basal zone there may be intercellular bridges, giving the appearance of prickle cells found in the epidermis. In the tunica propria are many leukocytes, especially lymphocytes. Smears from the vagina of some rodents show a series of changes, and the type of cells cast into the lumen indicates the progress of ovulation. Some authors recognize a submucosa below the tunica propria as a connective tissue of looser texture between it and the muscularis. The connective tissue of the adventitia connects the vagina with surrounding structures.

The Vestibule.—The vestibule is a shallow depression posterior to the vagina. Its surface is covered with stratified epithelium, which rests on a tunica propria of fibroelastic connective tissue. Below the tunica propria are muscles of the skeletal variety. A pair of vascular bodies form the clitoris adjacent to the entrance of the vagina. This is a structure similar to the corpora cavernosa of the penis and functions as erectile tissue in periods of sexual excitation. Several small glands are also present in the connective tissue around the opening to the vestibule, and their columnar cells secrete a mucous substance. The Bartholin glands which occur on either side of the vestibule may be homologous with the bulbourethral of the male.

ŒSTRUS.

In describing the ovary we followed the development of ova from the primitive germ cells to the time of their liberation, noting that such ovarian activity varied considerably in different forms. Usually there is a regular cyclical appearance of ovulation; in some forms it occurs annually, but in others it occurs at shorter intervals. Among the lower aquatic vertebrates, as in the fish, the mature eggs are discharged into the water during a spawning season, at which time males and females congregate. Copulation does not occur, but sperm are discharged into the water in the vicinity of the eggs so that fertilization and further development are external.

In other animals, as the frog, fertilization is also external, but is aided by a closer proximity of the male and female in an embrace called amplexus, a pseudocopulation. When the females become turgid with mature eggs, the male clasps a female tightly and as the eggs are discharged sperm are poured over them. Among the reptiles, birds, and mammals, sperm are introduced into the female cloaca or vagina, and fertilization later occurs in the oviducts. Most vertebrates, including the lower animals, show mating interest at the time of ovulation, and their outward behavior is generally influenced by this internal condition. There are some accompanying changes in the oviducts and uterine regions. In the lower forms these changes involve an activity of the glands adding albumen and shells to the eggs, but in those forms where development is internal there are additional preparatory changes in the uterus.

In most of the lower mammals, ovulation occurs during the period known as heat, rut, or œstrus. In the rat, cycles of heat, or œstrus, occur every four days, in the guinea-pig every sixteen days, in the pig every twenty-one days unless fertilization occurs. In each of these periods there is some uterine preparation for ovulation and also changes in the vaginal condition. The œstrus cycles in these forms can be followed by changes in the vaginal cells to the time at which the follicle is mature, at which time the animal is said to be in heat. A desire for mating is manifested at this time, and at the time of or following copulation, ovulation occurs. Should copulation not be effected, ovulation usually occurs, the egg degenerates, and the cycle is begun anew with the maturing of other follicles. In man and the primates the main evidence of a regular œstrus cycle is the phenomenon known as menstruation, which occurs with the failure of fertilization. In these forms the uterine preparation for fertilization is much more extensive and involves a remarkable thickening and softening of the mucosa of the body of the uterus for reception of the fertilized egg. In case the egg is not fertilized before it reaches the uterus such preparation is not utilized, and there follows a loss of the major portion of the uterine mucosa together with considerable blood. Menstruation represents a failure of the reproductive process and is followed by regeneration and a return to the resting stage before there is a renewal of preparation for the next ovulation. Maturation of follicles usually ceases with fertilization and is renewed after birth of the young.

MENSTRUATION.

In humans, ovulation occurs about every twenty-eight days. The changes undergone by the uterus may be indicated by separating this lunar period into four parts, understanding, of course, that the tissue changes are gradual and grade into each other. Beginning to count the days with the onset of menstruation, there are four stages:

Menstrual	First to fourth day.
Postmenstrual	Fifth to tenth day.
Intermenstrual	Eleventh to twentieth day.
Premenstrual	Twenty-first to twenty-eighth day.

The description already given for a resting uterus applies to the intermenstrual period. At the beginning of the premenstrual period the mucosa gradually increases in thickness until it is almost twice as thick as during the resting condition. (Figs. 142, 143, 144.) The glands become longer, assume a twisted course, and may branch externally. Droplets of glycogen and lipoid material develop in the cytoplasm of the gland cells, and the lumen of each gland becomes distended with secretion. The cells in the connective tissue immediately below the epithelium increase greatly in size. Capillaries in the tunica propria enlarge and are filled with blood to the point that their walls begin to give way and blood issues forth to collect in pools beneath the epithelium. The glands also begin to rupture laterally, and their secretion mingles with the escaped blood. The surface epithelium eventually can no longer withstand the distention, due to the continued collection of subjacent fluids, and begins to give way at numerous points. With the ruptures in the epithelial covering, the underlying fluids pour into the lumen of the uterus, carrying along small masses of epithelial tissue, mouths of glands, and tunica propria. Eventually the entire spongy internal lining of the uterine mucosa is sloughed off. Muscular contractions in the uterine wall begin during this period and continue until material accumulated in the lumen is expelled.

The postmenstrual period begins with the closure of the small blood vessels and cessation of loss of blood. Epithelial cells from the broken ends of the uterine glands migrate out to the surface, where an epithelial lining is reformed. The glands are likewise reformed and the connective tissue is restored to its former condition, so that by about the tenth day following the beginning of menstruation the uterine mucosa is entire again. The uterus then enters

into the intermenstrual, or resting period, during which time there are few changes until at the beginning of the premenstrual period, when renewed preparations are made for reception of the fertilized egg. Sometimes between two menstrual periods, generally
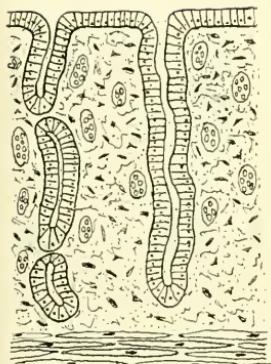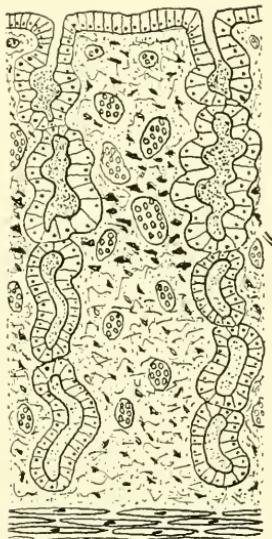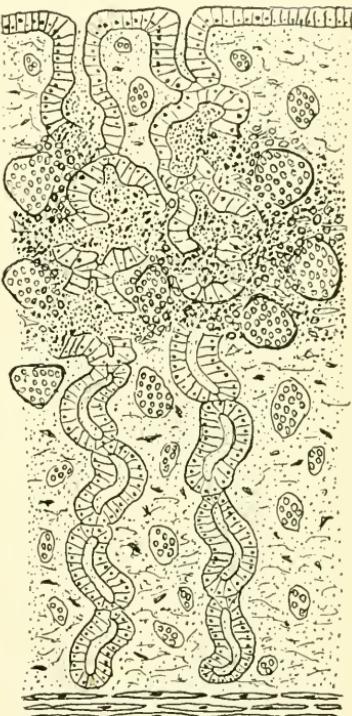
FIG. 142   FIG. 143   FIG. 144

FIGS. 142, 143 and 144.—Three diagrams illustrating changes in mucosa of the uterus during premenstrual period.

FIG. 142.—Below is the myometrium. In the mucosa are simple tubular glands lined with simple columnar epithelium. The tunica propria is fibrous, with many connective-tissue cells and also leukocytes. The mucosa is also very vascular with many venous-like capillaries.

FIG. 143.—The mucosa increases in thickness; the glands become elongated and more tortuous. Somewhat below the internal surface, connective-tissue cells at a certain level become much enlarged and are known as decidual cells. The capillaries increase in size. The epithelial cells in the zone of decidual cells enlarge and secrete the mucus collecting in the adjacent lumen.

FIG. 144.—The mucosa is much thicker (possibly twice as thick as in Fig. 142.) The decidual cells are more prominent. Epithelial cells of the glands in the neighborhood of outpocketings filled with secretion give way; fluid seeps out from capillary spaces in this region—the capillary walls give way and the mucous secretions and blood mingle in pools. The process continues until there is a general sloughing off of the internal lining of the mucosa. Half way through this menstrual (sloughing) period—the mucosa internal surface is lacking in epithelium. Later epithelial cells from ends of glands still left in deeper portions of the mucosa migrate out to the internal surface and organize a new lining and also outlets for the glands. It will be noted that the above mucosal changes do not involve the deeper portions of the glands as much as external portions adjacent to the lumen of the uterus.

accepted now to be about the tenth to the thirteenth day following the onset of menstruation, a follicle ruptures and discharges an ovum. Upon entering the oviduct the ovum undergoes its final maturation division and is fertilized if sperm are present.

PREGNANCY.

Following fertilization, development begins and continues as the egg passes along the oviduct toward the uterus. During this passage, which takes about three days, a number of cells are formed. The embryo comes to rest in some pocket or fold of the uterine mucosa and continues its development. While the uterus develops to a condition of the premenstrual period, the embryo develops the fetal membranes, and the chorion with tuft-like projections begins to form. This extremely small globe erodes its way into the uterine mucosa and, with development, establishes an intimate contact with the uterus through the placenta. To understand the relationship between the developing embryo and the uterine wall, the student is referred to embryological texts for developmental details of the fetal membranes.

Placenta.—Among the marsupials, as in the opossum, gestation, or uterine development of the young is brief and the young are born relatively immature. In these cases the chorion is a smooth membrane in close contact with the vascular uterine mucosa. The outer face of the rudimentary yolk sac unites with the inner face of the chorion, and thus food compounds of the maternal blood vessels in the uterine wall are in juxtaposition with those in the membranes of the embryo, and these food compounds pass into the chorionic vessels to be transported *via* yolk stalk vessels to the embryo. Oxygen is obtained similarly. Excretions pass from embryonic to maternal vessels. At the end of its uterine development the membranes surrounding the embryo pull loose from the uterine mucosa and the young is born with little destruction of the uterine mucosa.

In higher mammals the chorion develops branching vascular villi that penetrate and erode the uterine mucosa to establish varying degrees of intimate relationships. Such associations of embryonic and maternal tissues result in formation of an organ called the placenta where nutritive, respiratory, and excretory exchanges are carried on. The uterine mucosa has in the meantime grown over the entire surface of the chorion. Although the placenta varies

in form in different mammals, it never involves the entire outer surface of the chorion. As the embryo and its membranes grow, the amniotic sac and surrounding chorion covered with an overgrowth of uterine mucosa bulge into the uterine cavity. That part or aspect which from the beginning has been in closest relation with the uterine wall will be the site of the formation of the placenta. The placenta in humans is a disc-like plate, consisting of two components, one chorionic and one derived from the uterine mucosa. It is also termed the decidua basalis. The uterine mucosa which grows over the chorion of the embryonic mass, where it fills the uterine cavity, is known as the decidua capsularis and is continuous with the decidua basalis, or placenta. The remaining uterine lining also shows a mucosal thickening and is known as the decidua vera. Branching tuft-like outgrowths from the chorion invade the mucosa of the decidua capsularis and basalis. The chorionic villi of the decidua basalis are much more elaborate, and it is here that fetal nutrition, respiration, and excretion are effected.

Chorionic Villi.—The epithelium covering of the chorion and its villi is a syncytium, and from the underlying mesenchyme connective tissue develops carrying blood vessels. These villi become much extended until only a very thin layer of epithelium separates the fetal blood vessels from the blood in the sinuses of the uterine mucosa into which the villi project. The old superficial uterine wall epithelium disappears, although the superficial connective tissue is still quite firm. The deeper portions of the chorionic villi of the basalis lie in pools of blood in the deeper zone of the uterine mucosa, where the blood has escaped from the broken vessels, as described in the premenstrual condition of the uterus. The villi are covered with a syncytial epithelium which becomes thinner with greater expansion during embryonic growth. Within the epithelial covering of each villus is a connective-tissue core, containing two small arteries, veins, and capillaries. These vessels connect with those in the umbilical cord which arise from the center of the placenta opposite the surface associated with the uterine mucosa. The umbilical cord has a jelly-like connective tissue which encloses the two umbilical arteries, the umbilical vein, the allantoic stalk, and the rudimentary yolk stalk. Nutritive material and oxygen pass from the blood in the uterine pools through the epithelial membrane of the chorionic villi into the capillaries, which pass it through the umbilical vessels to the fetus.

The fetus and its membranes grow rapidly until the uterine cavity

is filled and the decidua capsularis fuses with the decidua vera. At birth, the amnion sac is usually torn open, the contained liquid flows down into the vagina, and the young is expelled by the powerful contractions of the hypertrophied myometrium of the uterine wall aided by contraction of the abdominal skeletal muscles. After the young emerges there is an after-birth. This consists in the expulsion of the amnion and the deciduæ, together with a considerable superficial portion of the hypertrophied uterine mucosa. This process involves considerable hemorrhage in case of intimate relationship between the chorionic villi and the uterine mucosa. After the birth process is completed, the hemorrhage usually ceases and there is a slow and gradual regeneration of the uterine mucosa, returning to a condition similar to that found in an ordinary intermenstrual, or resting period.

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See Appendix for general text references.

CHAPTER XIV.

THE MALE REPRODUCTIVE SYSTEM.

In the male vertebrate the organs of reproduction generally
consist of a pair of testes, in which spermatozoa are produced;
ducts by which the sperm are carried away from the testes; and
glands, whose secretions are added to the sperm passing through
the ducts.

DEVELOPMENT OF THE TESTES.

As in the case of the developing ovary already described, there
is an indifferent stage of gonad development during which both
testis and ovary cannot easily be distinguished. Relatively early,
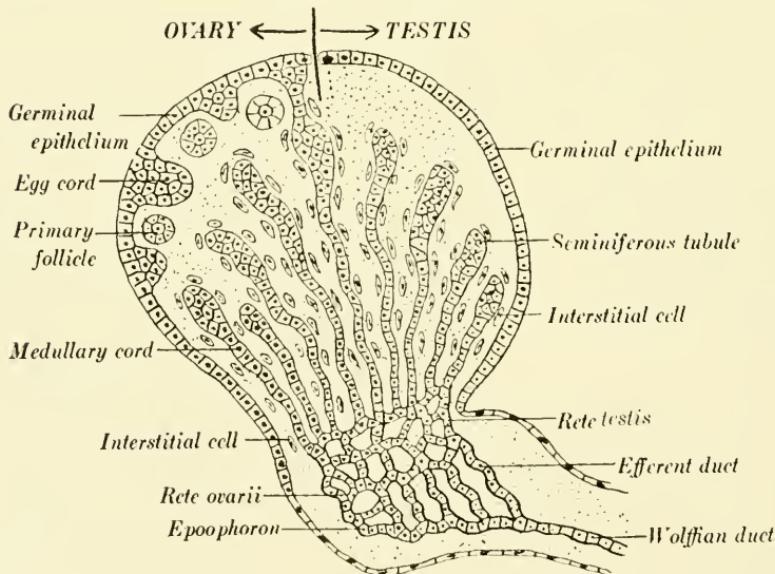
Fig. 145.—Diagram of the development of the testis and ovary.
(Adapted from Kohn.)

however, there is the beginning of differential development leading
to formation of either male or female organs of reproduction.
(Fig. 145.)

The developing cords of cells, which in the ovary broke into

numerous follicles, continue unbroken in the testis to form semin-
iferous cords in which a lumen later occurs and seminiferous tubules
result.   The mesenchyme between the tubules forms thin walls
about them and also septa separating the organ into compartments
called lobules.   The septa connect with the sheath of connective
tissue surrounding the testis.   (Fig. 146.)

When the early epithelial mass forms cords of cells, two types of
cells become distinguishable.   Of these, the greater number are
small polyhedral or cuboidal, and among them are fewer larger,
more or less spherical primitive sex cells with large nuclei.   Some
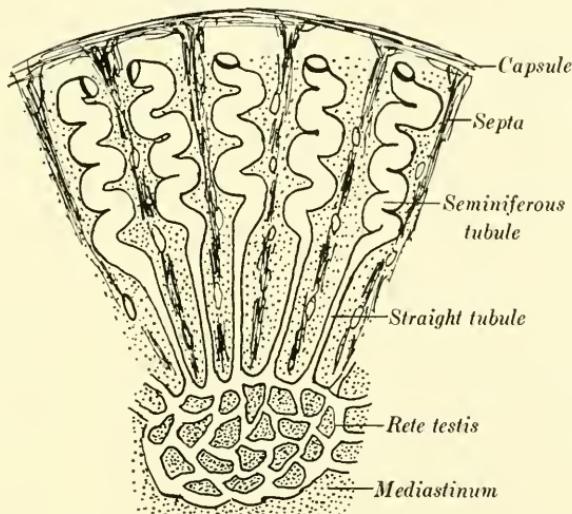
FIG. 146.— Diagram of the seminiferous tubules and rete testis of a mammal.

observers correlate the small cells with the follicle cells surrounding
the ova in a developing ovary and the large cells with the ova.

When the testis is mature, two types of cells, namely, spermato-
togonia and Sertoli cells, are the main components of the tubules.
Spermatogonia, which will give rise to spermatozoa, are believed to
be descendants of the large primitive sex cells, and the cells of
Sertoli are thought to develop from the small cells corresponding to
ovarian follicle cells.   However, there are repeated cycles of cell
degeneration and proliferation in these seminiferous tubules, and,
according to some investigators, the early sex cells are lost in the
process and the spermatogonial cells of the functional testis are
derived from the original small follicle cells that survive.   In either
case, spermatogonial cells alternate in location with Sertoli cells

in the walls of the mature testis tubules and are much more numerous. (Fig. 147.) The process of sperm cell formation from the spermatogonial cells is called spermatogenesis. Before maturity of the animal there is a series of abortive efforts to carry through development of sperm to completion, until finally the process is well established and fully differentiated and functional sperm are regularly produced.

Testes, like ovaries, are not always active but show seasonal periods whose duration and occurrence varies with the species. Both testes and the sperm ducts show variations, depending on whether they are studied during or between breeding seasons. During the inactive stage following or preceding a breeding season, most of the spermatogonial cells have degenerated, the lumens of the tubules are lost, interstitial connective tissue between the adjacent tubules increases in amount, and cords or spheres of large resting spermatogenic and Sertoli cells are prominent. The conditions at these times resemble those of the immature or developing testis before spermatogenesis has begun. To study the active condition it is necessary to secure the testis of an animal during the breeding season.

The vertebrate testis is usually a compound tubular gland, and the seminiferous tubules, where spermatozoa are formed, represent the secretory end-pieces. They are connected with a duct system by which the sperm pass to the outside.

SPERMATOGENESIS.

In general, spermatogenesis shows little variation among vertebrates, the series of divisions of cells and differentiation of the sperm presenting similar stages. The wall of each seminiferous tubule consists externally of circularly and longitudinally disposed collagenous and elastic fibers and connective-tissue cells. Between adjacent tubules there are regions filled with interstitial tissue composed of loose fibroelastic connective tissue and certain special elements, called interstitial cells, thought to be secretory. Within the connective-tissue sheath of each tubule is a basement membrane on which rests the highly specialized stratified germinal epithelium composed of Sertoli cells and spermatogonial cells. (Fig. 149.)

Sertoli Cells.—These are long columnar-like cells with broad bases resting on the basement membrane, with the elongated portions of the cells extending like spokes of a wheel part way toward the center

of the lumen. The nucleus is oval and somewhat elongated. Sertoli cells occur at quite regular intervals around the lumen of the tubule and establish close relationship with the more numerous and smaller developing spermatogenic cells.
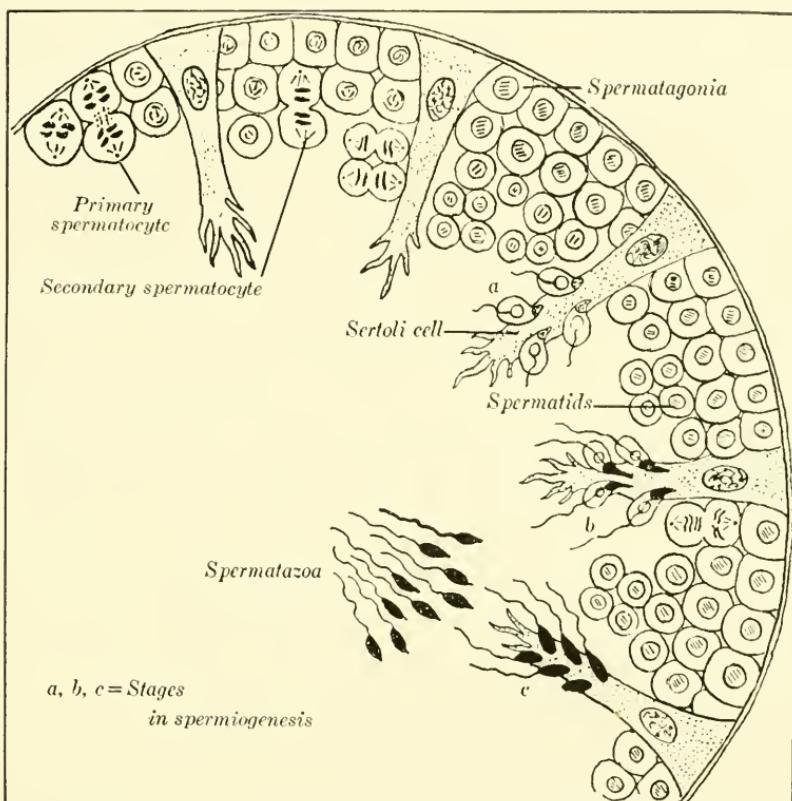
FIG. 147.—Diagram of a section through a seminiferous tubule, showing formation of spermatozoa from spermatogonial cells.

Spermatogenic Cells.—Beginning at the basement membrane, and extending in toward the lumen, spermatogenic cells form a stratified epithelial tissue several layers deep, interrupted at regular intervals by Sertoli cells. We have already studied the growth of stratified squamous epithelium, and the activity of these spermatogenic cells presents something of the same sort, but of a more highly specialized nature. When activity begins which will ultimately lead to the formation of sperm, a series of mitotic divisions take place, during which daughter cells of the dividing basal cells are constantly pushed

inward toward the lumen. A reduction of the chromosome number from diploid to haploid is effected, and then a differential process takes place as a result of which sperm are produced. The phases of development up to and including reduction of the chromosome number constitute spermatocytogenesis, and the following stage of differentiation of the more or less spherical cells with haploid chromosome number into spermatozoa is called spermiogenesis. Not all the tubules or all portions of any one tubule are actively producing sperm at any one time. In an active tubule, there are rounded cuboidal spermatogonial cells resting on the basement membrane and undergoing mitosis. As each divides, one daughter cell is pushed toward the lumen and in turn it divides so that one of the daughter cells is again pushed further toward the lumen. The number of mitoses, or spermatogonial divisions, appears to show some variation in different animals, but the cells resulting from the last mitosis of this early proliferation period are known as *primary spermatocytes*, and they enter a resting phase during which they increase in size. Later they undergo a maturation process involving a reduction division and a following regular mitotic division which produces the spermatids, small spherical cells with a haploid chromosome number.

After spermatids are formed they associate themselves closely with the tapering distal portions of the Sertoli cells. As a result of a transformation process, these small spherical cells become fully developed spermatozoa, with head, middle-piece, and tail. The head is composed of the condensed nuclear matter of the haploid number of chromosomes; the middle-piece immediately behind the head contains some of the cytoplasm and the centrioles; and the tail is a long flagellum-like process. Much of the cytoplasm of the spermatid is sloughed off during differentiation of the sperm and discharged into the lumen where it may join the sperm in their passage toward the efferent ducts or be resorbed in part by the Sertoli cells. During the process of spermiogenesis the head remains buried in the Sertoli cell and the tail when formed extends into the lumen of the tubule. When fully formed the sperm become free of the Sertoli cells and pass into the lumen, but are themselves as yet quite inactive. The sperm show some variations with regard to size and shape, but the parts are fundamentally very similar.

If a particular section of a seminiferous tubule shows activity, a number of cells appear to be in the same stage of development. If spermatids are present, a number in the same stage appear; similarly only spermatocytes in the same phase, or only spermato-

gonia may be apparent. If one crop of spermatids is completing metamorphosis into sperm, then mitotic activity may already have begun in the spermatogonia initiating another group of spermatids. In many mammals it appears that spermatogenic activity passes along the seminiferous tubule in waves from their distal ends toward the efferent ducts. Spermatogenesis has been intensively studied in the rat, where it has been estimated that it takes about twenty days for a spermatogonial cell to develop into mature sperm.

This spermatogenic activity is limited to animals that have reached the age of maturity. Breeding seasons usually occur during this period of activity.

Interstitial Cells.—Between the adjacent seminiferous tubules is a loose fibroelastic connective tissue similar to that forming the wall of the tubules. In this tissue region are the blood and lymph vessels, nerves, and so-called interstitial cells. (Fig. 149.) These cells appear to have about the same size as the granular leukocytes and show variation in shape. If they are connective-tissue cells they appear to be a special variety and their activity is thought to be endocrine in nature. The spherical nucleus is relatively large, with coarse chromatin granules and one or two nucleoli; the cytoplasm shows variation in the degree of granulation of a fatty nature. Transitional forms indicate the origin of these cells from fibroblasts and their return to a fibroblast type under conditions of inflammation. The true nature of these cells still remains undetermined. Endocrine secretion on the part of the testis is evidenced by the effects following its removal and transplantation. For example, if the testes are removed from an immature animal, the secondary sexual characters of males do not develop; if this removal takes place after maturity there is a loss of sex emotions and reproductive activities, and the body accumulates considerable adipose tissue. The transplantation of a testis into the body of a female causes secondary sexual characters of the male to make their appearance in the absence of the ovaries. A number of such experiments point to an endocrine activity on the part of the testis, but do not indicate the tissue directly responsible.

SPERM DUCTS.

In studying the urinary system attention was called to the fact that in the male parts of the mesonephric tubules became associated with the testis as efferent ducts for the sperm. At the same time the seminiferous tubules are developing in the testis, cells of

the anterior mesonephric tubules proliferate to extend as cords into the region of the developing seminiferous tubules. Such mesonephric derivatives unite with the cords forming in the testis, both develop lumens, and the numerous seminiferous tubules acquire an outlet through the modified mesonephric tubules, now called vasa efferentia, to the Wolffian duct. The whole anterior end of the Wolffian duct may become much convoluted to form the epididymis, a special part of the duct system, to be described later. In the amniotes the entire Wolffian duct is no longer urinary, but becomes the vas deferens, or main sperm duct, of the male. Variations exist through the ascending groups as regards the completeness of a separation of the two systems. In cyclostomes the sperm may have no duct system, but pass directly to the cœlome and out through abdominal pores; in mammals the sperm pass through the converted mesonephric duct system independent of the urinary system until it joins the urethra, in the vicinity of the copulatory organ, the penis.

In general, the sperm ducts are lined with a cuboidal epithelium where they unite with the seminiferous tubules, but approaching the posterior portions the epithelium changes to columnar, and in the vas deferens it may become stratified columnar. Ciliated cells often alternate with those of glandular types whose secretions enter the lumen and join the sperm. The epithelium rests on a connective-tissue sheath encircled by scattered smooth muscle cells in the smallest ducts and a definite coat in the vas deferens. Surrounding the ducts peripherally is a vascular fibroelastic connective tissue which becomes more prominent as the size of the duct increases.

MALE REPRODUCTIVE SYSTEM OF FISHES.

In elasmobranchs the testes are ovoid or cylindrical bodies. The mesonephros is the functional kidney, and in the male the anterior end of it degenerates so far as its urinary function is concerned, and its ducts establish connections with the tubules of the testis during development. Some of the embryonic mesonephric tubules of the anterior end form a much-coiled tube, the epididymis, through which sperm pass from the testis to the mesonephric, or Wolffian, duct, which serves to conduct both urine and sperm to the cloaca. In some cases the kidneys empty by special urinary ducts into a sort of urogenital sinus from which a papilla, the urogenital papilla extends into the cloaca. The posterior ends of the two Wolffian ducts are enlarged and act as sperm reservoirs during the breeding season.

MALE REPRODUCTIVE SYSTEM OF AMPHIBIA.

The testes are more or less elongated, tubular bodies in the urodeles and lower forms, and ovoid in anurans. They are ovoid, yellowish-white bodies lying alongside or ventral to the kidneys and connected to the latter by a mesenteric sheet of tissue. Each testis is a mass of tubules fitting the general description given for the seminiferous tubules. The seminiferous tubules connect with the vasa efferentia, derived from embryonic mesonephric tubules and supported by mesentery. When the efferentia reach the functional kidney they pass into a longitudinal duct, Bidder's canal, which runs along the mesial border of the mesonephros. (Fig. 148.)
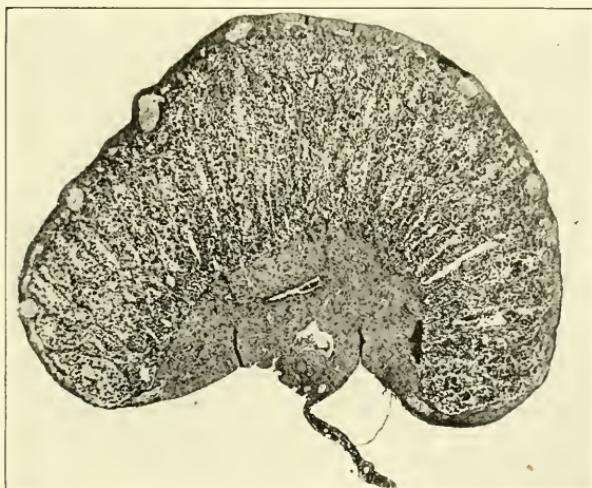
Fig. 148.—Longitudinal section of testis of Necturus, showing seminiferous tubules radiating toward ducts in connective tissue below.

During development of anurans, an anterior part of the testis, possibly derived from pronephric tissue, has large cells resembling oöcytes or enlarged spermatocytes. This structure, Bidder's organ, may persist well into the mature stage of toads, but gradually degenerates. The remains of the pronephric ducts passing along the mesonephros form Bidder's canal. Its lumen is lined with columnar epithelium supported by a connective tissue and a few smooth muscle cells. The outermost region of the canal has connective tissue continuous with that of the covering of the kidney in which it is partially embedded.

Bidder's canal communicates with the collecting tubules in the

16

kidney and the ureter which runs along the outer margin of the kidney. Thus, in the breeding season the ureter, or Wolffian duct, acts as a sperm duct also. The testis shows seasonal variations in size, being largest during the breeding season.

In many Amphibia, the Müllerian duct, which is the functional oviduct of the female, is still present in the male. It may be without a lumen in some, but in others it has the features of the resting oviduct of the female.

MALE REPRODUCTIVE SYSTEM OF REPTILES.

In reptiles and birds the mesonephros is an embryonic structure, but is replaced by the metanephros, which is the functional kidney of the adult, and ureters are new developments from the embryonic cloaca. Each testis has associated with it an epididymis, a coiled sperm-carrying duct, distally connected with the seminiferous tubules and proximally connected with the Wolffian duct. The Wolffian duct has become a vas deferens and is now solely a sperm-carrying duct. In some male reptiles the Müllerian duct still remains, and in Lacerta viridis is as large as in the female, where it is the oviduct. In the epididymal region the duct is lined with cuboidal or columnar epithelium, which may be ciliated; in the vas deferens the epithelium is columnar or may appear stratified with regions of glandular cells alternating with ciliated cells.

MALE REPRODUCTIVE SYSTEM OF MAMMALS.

Among the mammals the testes are paired, ovoid, compact organs which show some variation in their location, not only in different species, but often in the same species at different periods. In such animals as elephants and whales the testes remain permanently within the body cavity, but in marsupials, rodents, bats, and some insectivora they pass out of the body into scrotal sacs during the breeding season. In primates, carnivora, and ruminants they remain permanently outside in scrotal sacs.

In the testes that have descended during the breeding season, or in the case of primates where they are permanently outside the body cavity, the scrotal sac adds several tissue sheaths about the testes. The scrotal sac, or scrotum, is formed by an evagination of the body wall. Externally it has a covering of skin with its stratified squamous epithelium, glands, and hairs, supported by layers of connective tissue, and muscle. Internally it is lined with a tissue similar to the peritoneum and known as tunica vaginalis parietalis. The same type of tissue is reflected over the anterior and lateral

faces of the testes and somewhat ventrally and dorsally as the tunica
vaginalis visceralis.   Between these two tunics is a very loose fibro-
elastic connective tissue and fluid, making possible a free movement
of the testis within the scrotum.   Within the tunic vaginalis, a
connective-tissue sheath, the tunica albuginea, extends perforated
partitions, or septa, into the testis and forms compartments or
lobules containing the seminiferous tubules.   A mid-posterior region
of this tissue located toward the surface of the testis is very vascular
and called the mediastinum.   The lobules so set off in the testis
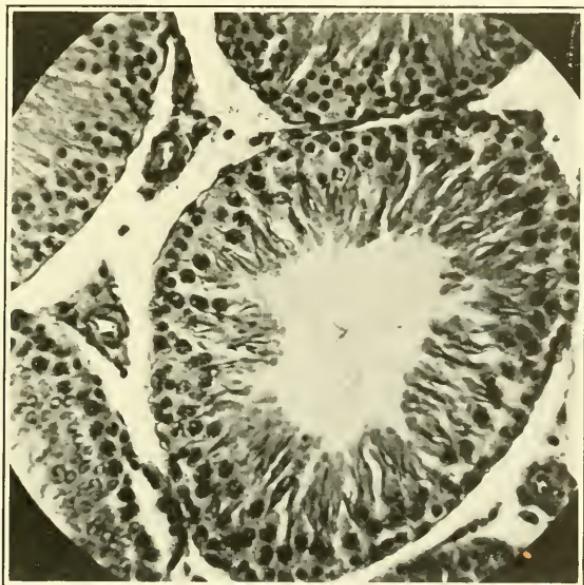
Fig. 149.—Photograph of a cross-section of a seminiferous tubule of the woodchuck
showing interstitial tissue between it and adjacent tubules.

contain from several to many convoluted seminiferous tubules.
(Fig. 149.)   As the tubules approach the mediastinum they unite
to form less-coiled tubules until a few much smaller straight tubules
are formed which connect within the mediastinum into a network
of small ducts called the rete testis.   From the upper region of the
rete testis a number of larger tubules compose the vasa efferentia,
which are at first straight but become twisted and increase in diam-
eter as they pass toward the upper posterior region of the testis.
These join to form the epididymis, a much-coiled duct forming a
mass divided into a head and tail portion at either end of the testis,
and the body of the epididymis along the central portion.   The
tail portion of the epididymis straightens out to become the vas

deferens and passes upward through the inguinal canal into the
body cavity with the spermatic cord. The spermatic cord is com-
posed of connective tissue containing the vas deferens, the spermatic
artery, spermatic vein, the nerve trunk, and a plexus of veins, called
the pampiniform plexus. After entering the body cavity, the vas
deferens continues anteriorly for a distance, then turns down
around the ureter to join the urethra somewhere along its passage
from the bladder to the exterior. Each vas deferens has an enlarge-
ment, called an ampulla, near its terminal region in the case of
primates, shrews, bears, dogs, and most rodents. In some, the
walls of this region have mucoid glands. (Fig. 150.)
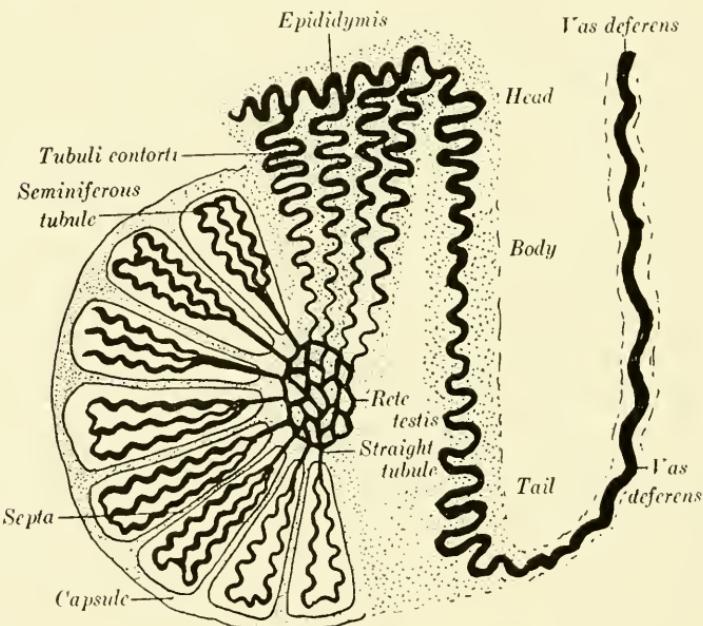
FIG. 150.—Diagram of the tubular system of a mammalian testis.

Connected with each vas deferens in the ampullar, or terminal,
region is a saccular organ, the *seminal vesicle*. These occur in most
mammals, but not in marsupials and carnivora. The neck of each
ampulla and each seminal vesicle unite to form the *ejaculatory duct*.
There is a large globular mass known as the *prostate gland*, which
surrounds the urethra near the bladder, and each ejaculatory duct
traverses the prostate to join the urethra. The prostate contains
a number of small glands and occurs in most mammals, but not in
marsupials. A short distance along the urethra toward the exterior

is a pair of small sac-like structures, known as the bulbo-urethral, or Cowper's glands, which are present in almost all mammals, but not in dogs and bears. They are large in rodents and pigs.

Having already considered the histology of the seminiferous tubules, let us now study the other parts of the conducting system.

Tubuli Recti.—Where the seminiferous tubule transforms into a straight tubule, the spermatogonial cells are lacking, and the straight tubules are lined with columnar cells resembling the Sertoli cells of the tubules.

Rete Testis.—The irregular meshwork of these spaces is lined with short cuboidal or squamous epithelium. The free surface of the cells possesses a flagellum. In between the rete testis is connective tissue, nerves, and vessels of the mediastinum.

Vasa Efferentia (Tubuli Contorti).—The cells lining the ducts rest upon a basement membrane and vary in size. A clump of tall, ciliated columnar cells may have adjacent to them progressively shorter cells. Thus, little pockets are formed in the wall of the efferent ducts. At the free surface of the short type of cell is a flagellum. The cells of these tubes produce a secretion which is added to the mass of sperm moving through the ducts to the epididymis. In the connective tissue outside the basement membrane there is a small amount of smooth muscle.

Epididymis.—The lumen is larger and circular in cross-section. Adjacent to the basement membrane are small cells and internal to them are large columnar cells of varying heights. At the free surface of each of the latter cells is a filamentous projection that is non-motile but from which secretion originating from the cytoplasm of the cells is discharged into the lumen. As the epididymis approaches the vas deferens the columnar cells are not so tall. In the connective tissue outside the basement membrane there are smooth muscle cells, which constrict the tube and serve to propel sperm and fluid onward toward the vas deferens. The rete testis, tubuli contorti, and epididymis are portions of the old mesonephric tubules used here exclusively to carry sperm.

Vas Deferens. (Fig. 151.)—This is a larger tube than the epididymis and its lumen is also greater. The epithelium of its mucous membrane is of the stratified columnar variety. The tunica propria has long folds which make the lumen irregular in shape. There is no definite submucosa, but outside the tunica propria is a muscle coat which consists of a thin innermost coat in which the smooth muscle cells are arranged lengthwise of the tube, a relatively thick middle circular coat of muscle, and an outer longitudinal coat which

is thinner than the circular coat.   Outside the muscle coat there is
an adventitia of fibrous tissue in which there are groups of smooth
muscle cells.   As the vas deferens continues into the body cavity,
a connective-tissue extension of the adventitia supports the sper-
matic artery, spermatic vein, nerves, and a plexus of veins, called
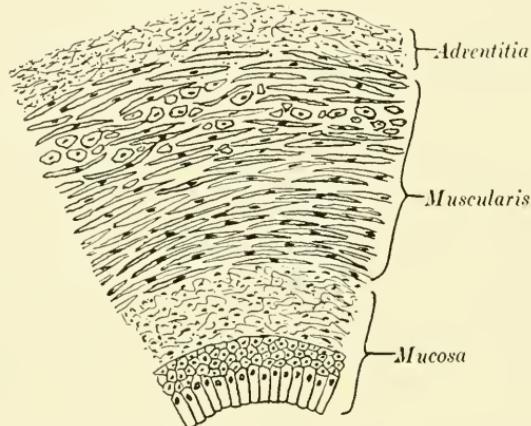
Fig. 151.—Diagram of the vas deferens of a mammal.
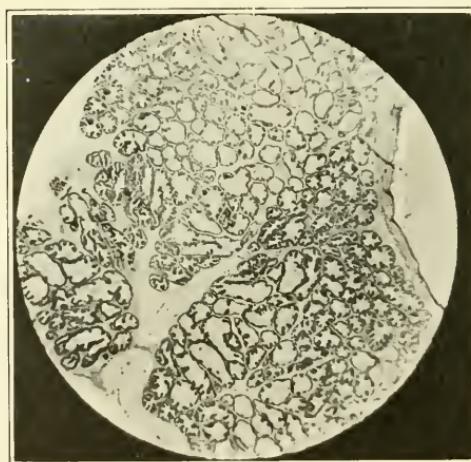
Fig. 152.—Photograph of a section through the seminal vesicle of the rat.

the pampiniform plexus, the whole being called a spermatic cord.
The ampulla at the distal end of the vas deferens has large ridges
in the tunica propria, and in its walls are glandular outpocketings.

Seminal Vesicles.—Each seminal vesicle is an elongated, lobulated,
twisted irregular sac, with an adventitia continuous with that of

the vas deferens. Within the adventitia is a thin muscularis coat
with an outer longitudinal sheath. The tunica propria of loose
connective tissue supports an epithelium of simple columnar epithe-
lium or of cells of the same type as the vas deferens, *i. e.*, stratified
columnar. Any cross-section reveals many complicated passages
and spaces set off from each other. (Fig. 152.) This is because the
mucous membrane is elaborately folded and in places the folds have
fused to form a complex system of labyrinthine passages within
the lumen. The seminal vesicles probably do not store sperm but
supply a secretion which is added to the sperm fluid proper after
the latter enters the urethra at the time of discharge. The lumen
is usually filled with an acidophilic colloidal material.

Fig. 153.—Photograph of a cross-section of the dog's prostate, showing 5 glandular
masses opening into the urethra by excretory ducts.

The Prostate.—The prostate is a glandular mass, roughly spherical
in shape, which surrounds the male urethra just distal to its origin
from the bladder. (Fig. 153.) Within the prostatic mass are a
number of small glands of the compound tubular and alveolar type.
There are a number of ducts from these glands entering the urethra.
Around the whole prostatic mass is a capsule of fibroelastic connec-
tive tissue which has extensions within the mass between adjacent
glandular structures. The epithelium lining the glands varies from
cuboidal to columnar. In the connective tissue between the
glandular portions are patches of smooth muscle cells indicating
that the mass may contract and force its secretions into the urethra.
The secretion is a thin, whitish fluid and forms the bulk of the
spermatic fluid. Sometimes spherical masses of varying size with
concentric lamellæ occur in the lumens of the glands.

Cowper's Glands.— Cowper's glands are also known as bulbo-urethral glands and are a pair of small masses located a little further along the urethra from the prostate. They consist of connective tissue, smooth muscle cells, and small branching compound tubulo-alveolar glands. The walls of the glands have small pockets and simple epithelium of different types lines them; in some places the cells are squamous, in others columnar. The main ducts are lined with transitional or stratified columnar epithelium. Other small glands occur along the course of the urethra.

COPULATORY ORGANS.

Lower Vertebrates. — In forms where fertilization occurs before the eggs leave the body of the female there is a structural modification in the male to facilitate the introduction of sperm into the female reproductive system.

In the male elasmobranch, each pelvic fin adjacent to the cloacal aperture has a long finger-like process called a clasper. These two structures are inserted into the cloaca of the female, and the spermatic fluid runs along the groove formed between the claspers. In some species of elasmobranchs, curved spines may be present at the outer end of the claspers. In most fishes, fertilization is external.

In the frog, the male embraces the female and, as the latter discharges eggs into the water, spermatic fluid is discharged from the male into the water about the eggs. A unique method is found in some urodeles, such as Amblystoma and Triton, where males discharge packets of sperm, called spermatophores, formed by the addition of secretions from glands in the cloacal wall. After these have been discharged, the female crawls over the sperm-packet until the swollen lips of the cloaca seize it and withdraw it into the cloaca.

In reptiles, a copulatory organ is present. On each side of the transverse cloacal aperture, among the snakes and lizards, there is a pocket in which an eversible sac lies. When these two sacs are inflated and everted they form a copulatory organ, called an hemipenis. On the surface of each sac is a groove, along which spermatic fluid passes when copulation with a female takes place. In crocodiles and turtles, there is a cloacal penis formed from the ventral wall of the cloaca. Under the epithelium of this region there is loose fibroelastic connective tissue containing large vascular spaces;

a subjacent region is composed of denser connective tissue and skeletal muscle. During the breeding season, when copulation is to take place, the vascular spaces become engorged with blood and convert it into erectile tissue that is thrust out from the cloacal opening. Between breeding seasons this structure lies collapsed within the cloaca.

The monotreme has a cloacal penis somewhat like that of reptiles and birds, except that the urinary passage, the urethra, runs through its center. This penis lies retracted in a cloacal sheath except during the breeding season.

Penis of Mammals.—In the body, or shaft, of the penis, masses of mesenchyme tissue give rise to corpora cavernosa or erectile tissue and also the cartilage or bone which forms in some species. The urethra passes only a short distance from the bladder before becoming associated with the ducts and glands of the genital system. Near the bladder it has a transitional epithelial lining, but this often changes to stratified columnar further along and then to stratified squamous at the opening. Three regions of the urethra have been separated. The prostatic portion extends from the neck of the bladder through the prostate gland, where ejaculatory ducts usually join it. The membranous, or middle part, is short, extending from the prostate region to the beginning of the corpora cavernosa of the penis. The cavernosa portion extends through the length of the penis. Along the course of the urethra are glandular outpocketings of the dorsal and lateral walls, already described, embedded in the fibroelastic connective tissue, with varying amounts of smooth muscle also represented.

A cross-section through the body of the penis shows that the major portion of it is formed by the corpora cavernosa. In man, two corpora cavernosa penis occupy the upper portion, but in other mammals they usually fuse to form an unpaired U-shaped mass. In man a third, corpus cavernosum urethra, surrounds the urethra and is located ventral to the other two (corpora cavernosa penis). In many mammals the urethral cavernosa tissue is poorly represented. (Fig. 154.) These corpora constitute the erectile tissue of the penis. Among the rodents, the vas deferens may not join the urethra until they reach the tip of the penis.

The corpora cavernosa are composed of a vascular sponge-like network with irregular venous sinuses capable of being filled with blood and distended under considerable pressure. Each of these bodies is surrounded by a thick fibrous membrane, the tunica

albuginea, with outer fibers mainly longitudinal and inner fibers circular. Between the two corpora this sheath forms a more or less complete septum, but toward the glans both corpora may communicate.

The corpus cavernosum urethra is surrounded by a less dense tunica albuginea, in which circular smooth muscle may appear toward the urethral wall. The venous sinuses are more uniform and more elastic fibers are present. When the os priapi, or penis
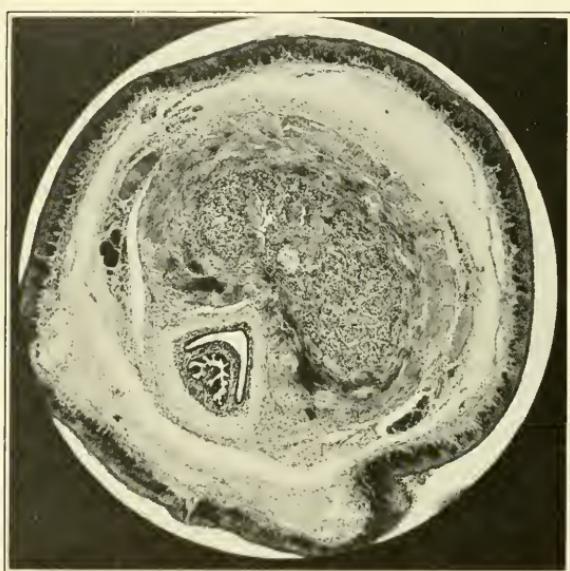
Fig. 154.— Photograph of a cross-section of the penis of a chipmunk near the tip. It is externally surrounded by the skin, within which is connective tissue. The corpus cavernosum penis is a large bean-shaped mass. The V-shaped opening is the urethra, below which is the vas deferens; both of these ducts are surrounded by scanty corpus cavernosum urethra tissue.

bone, is present, it forms just above the urethra from mesenchyme of the developing penis.

Surrounding the corpora and forming the general groundwork of the penis is a vascular fibroelastic connective tissue which is covered externally by the skin in whose subcutaneous tissue some skeletal muscle may be found. The outer knob-like end of the penis, the glans, is composed of a mass of dense vascular connective tissue covered by a reflected fold of skin, the prepuce.

At times of sexual excitement, erection of the penis follows reflexes conducted to the muscular wall of the arteries leading into

the cavernous tissue so that a relaxation occurs resulting in the supplying of an unusual amount of blood to the venous sinuses. The spaces of the cavernosa become engorged, so that the veins emptying them are compressed to the point of interfering with the outward flow of blood. Continued flow of blood into the sinuses enlarges the corpora under pressure and results in making the penis quite rigid. After completion of spermatic discharge or passage of excitement, the arterial walls regain their former tone, become constricted, and the inflow of blood diminishes. The emptying of cavernosa spaces is slowly effected; the penis slowly contracts and becomes relaxed.

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See Appendix for general text references.

CHAPTER XV.

THE ENDOCRINE GLANDS.

It is known that the nervous system controls integration of bodily activities so that there is a harmonious functioning of the organ systems making possible successful adjustments to changes in the external world. In addition, an important part of the unifying and coördinating function is effected by the system of chemical coördinators, the endocrine gland secretions.

The secretion products of *exocrine glands*, as already described, collect in ducts. *Endocrine glands*, on the contrary, have no excretory duct systems. Their secretions pass into the blood or lymph vessels and thus enter the circulation stream for distribution to various organs and systems.
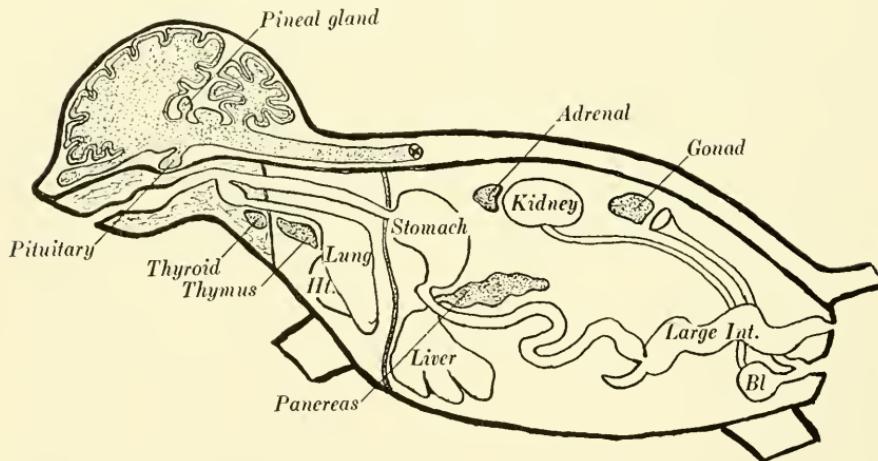
Fig. 155.—Diagram showing the location of endocrine glands.

An endocrine gland may be defined as one that secretes into the blood stream some hormone or chemical substance which stimulates or depresses the physiological activity of other groups of cells, thus affecting growth, development, and the general condition of the body as a whole. Secretion of one gland may affect the activity or effect of the secretion of some other endocrine gland, *i. e.*, they are physiologically interrelated.

Observations indicate that certain gland organizations are purely endocrine in nature. Among the outstanding endocrine organs are the thyroid gland, the parathyroids, the hypophysis cerebri or pituitary, and the adrenals. (Fig. 155.)

THE PITUITARY GLAND.

This name, literally meaning "phlegm," is said to have been devised by Galen (130-200 A.D.), since that ancient anatomist-physician thought that nasal secretions were produced by this gland. It is also called the *hypophysis cerebri, i. e.,* that which grows under the brain, and in mammals is located in a little pocket in the sphenoid bone on the floor of the skull posterior to the optic chiasma. The connective-tissue sheath of the brain, the pia mater, immediately surrounds it. The pituitary is connected with the lower end of the infundibulum of the brain. It is found in all
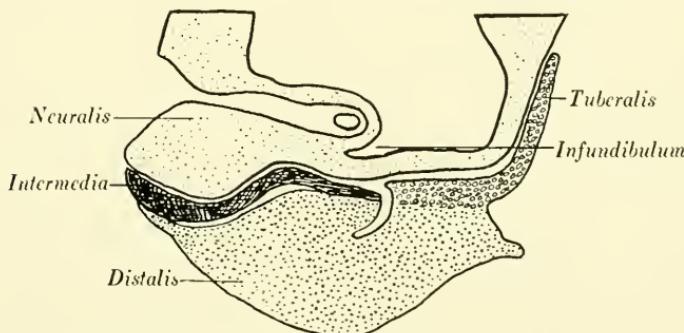
Fig. 156.—Diagram of the pituitary gland of a mammal. (After Atwell, Gray's Anatomy.)

the vertebrates, but its size in relation to the size of the entire body decreases in successively higher groups.

Four parts may be distinguished: an anterior lobe, or pars distalis; an intermediate portion, or pars intermedia; an upward forward part, the pars tuberalis; and a posterior lobe, the pars neuralis. (Fig. 156.) It has a double embryonic origin. An evagination of buccal epithelium (ectoderm) grows upward, forming a pocket, Rathke's pouch, toward the brain. At about the same time there is a downward evagination from the floor of the diencephalon, and Rathke's pouch comes into contact with it. These two components form the pituitary gland, Rathke's pouch forming the first three portions and the neural evagination forming the pars

neuralis. (Fig. 157.) The connection of Rathke's pouch with the mouth cavity disappears, and the original lumen of this pouch is eliminated, due to the great overgrowth of the anterior wall to form the pars distalis. Lateral lobe enlargements on either side unite with each other to form the pars tuberalis. The posterior wall of Rathke's pouch remains relatively thin and forms the pars intermedia. In some forms the pars intermedia grows around the pars neuralis. The original lumen in the downgrowth from the brain disappears in most forms.
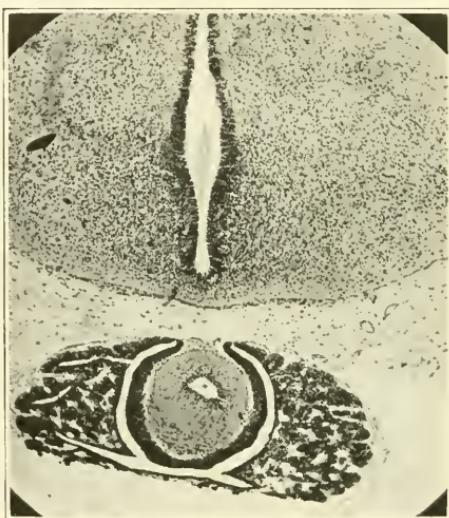
Fig. 157.—Photograph of a section through the diencephalon and pituitary of the kitten. The inner lighter portion of the pituitary is the neuralis and is surrounded by the intermedia. There is a space between the intermedia and the adjacent masses of the pars distalis.

The pars distalis (anterior lobe) of an adult pituitary is an epithelioid mass with cells arranged in cords. Three types of cells are recognized, namely, basophil cells, acidophil cells, and chromophobe cells. In higher vertebrates there are relatively more of the first two types. Between the cords and groups of epithelioid cells are sinusoidal capillaries. Around the outside of the lobe is a dense connective-tissue capsule from which extensions pass into the interior, separating the columns of cells and supporting the vascular supply.

The pars tuberalis resembles the pars distalis in structure. There may be incomplete follicular arrangements of cells with colloid

substance within the follicle, and patches of squamous epithelium left over from embryonic buccal epithelium.

The pars intermedia consists of epithelioid cells, some of which are columnar in type. Follicles may occur. In some cases the cleft between the pars intermedia and pars distalis has been obliterated, so that it is very difficult to note any histological difference between the two regions.

The pars neuralis appears to possess cells which are more like neuroglia than neurons. Some are spindle-shaped and some stellate with numerous processes. The fibers present are non-medullated. The cavity of the original embryonic infundibular evagination remains in the pituitary of the cat, but disappears in many other animals.

Although much positive information has been gathered concerning the functions of the pituitary, further research is necessary before it is completely understood. If the pars distalis is experimentally removed in young animals, they remain small in stature and the skeleton does not grow. Hyposecretion from this lobe causes diminished oxygen metabolism, dry skin, decrease of hair formation, short appendages, and dwarfism. Hypertrophy or hypersecretion in young individuals causes formation of more adipose tissue, larger bones, and gigantism. If the anterior lobe hypertrophies in adults, the bones and soft parts of the face, hands, and feet grow larger, a condition known as acromegaly.

The pars neuralis apparently produces a secretion which tends to cause a rise in blood-pressure, a modification in the volume of urine formed in a given time, and increased milk flow in active mammary glands. It is somehow related to the change of glycogen to glucose in the liver; it causes contraction of the smooth muscle of the bladder, intestine, and uterus. Because of this last-named property, extract of pars neuralis substance, or "pituitrin," is employed in obstetrics.

Removal of the entire pituitary from living animals results in a lowering of basal metabolism, lowering of body temperature, and in a short time even death.

THE THYROID GLAND.

In fishes this gland is a number of small follicular masses along the ventral aorta. In the frog, two masses are separated, each portion being located laterally in the floor of the mouth between the posterior lateral and thyrohyoid processes of the hyoid appa-

ratus. In higher vertebrates there is a tendency for a combination
of the two portions into one gland. In mammals the thyroid is
located below the larynx on the ventral surface of the trachea
adjacent to the upper tracheal cartilages. The form varies in
different mammals. In man there is an oval-shaped mass on
each side of the anterior face of the trachea connected by a median
"isthmus."

The thyroid begins its development as an evagination of cells
from the mid-ventral wall of the pharynx in the region of the first
pharyngeal pouches or somewhat posteriorly. This mass of early
cells, the thyroid "anlage," grows into a long stalk connected with
the mouth cavity at the base of the tongue, behind the papillated
region, and forms a distal larger mass which grows into the isthmus
of the mature gland. Later the stalk atrophies, thus shutting off
the "isthmus" from the digestive system. However, the site of
origin remains in the adult as the foramen cecum, a small depression
at the base of the tongue on its upper surface, just back of the most
posterior vallate papillæ. In addition to the above, lateral anlages
grow down from the lower surface of each fourth pharyngeal pouch
and establish a connection on either side with the isthmus to form
the lateral lobes

Connective tissue grows around the outside, forming an outer
loose sheath and an inner firm capsule of fibroelastic connective tissue
from which extensions are carried in between groups of gland cells.

In young embryos the gland cells arrange themselves in the form
of more or less spherical follicles resembling the terminal end-pieces
of an alveolar gland.

The mature thyroid consists of follicles which are structurally
independent of one another. (Fig. 158.) As they develop, a clear
yellow viscid colloidal fluid with an affinity for acid dyes collects
inside each follicle. The follicular wall is composed of cuboidal
epithelial cells, although the form varies somewhat with age, the
breeding season, and the type of food. Both mitochondria and
Golgi apparatus have been demonstrated in the follicle cells. The
inner border of these cells is cuticular, and there is no basement
membrane supporting them. The nuclei are large, round, and
regularly placed, and the side boundaries of the cells are indefinitely
indicated. Between adjacent follicles is loose fibroelastic connec-
tive tissue and a reticular network. The connective tissue supports
a very rich capillary network, so that the gland receives a relatively
large quantity of blood.

Histiocytes and lymphocytes occur in the interfollicular connective tissues and also nerves from sympathetic ganglia. Although some investigators regard colloid as a waste material, others are inclined to view it as a reserve secretion which passes out into the vascular network to find its way into the general circulation. It has been ascertained that the thyroid collects iodine from the blood and forms an organic compound, called thyroxin, which regulates basal metabolism. Deficiencies in thyroxin result in well-recognized abnormalities. Cretinism is due to failure of the thyroid
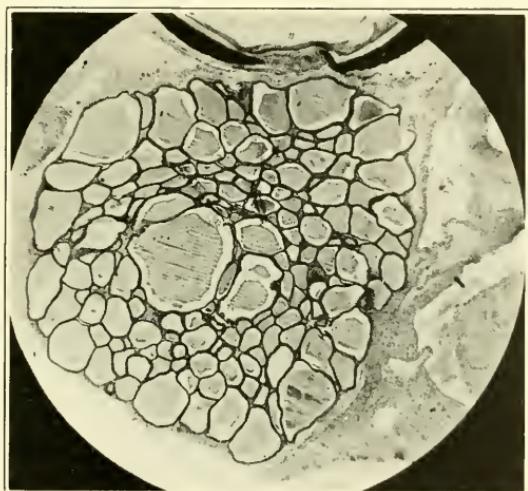
Fig. 158.—Photograph of a section through the thyroid of the water snake, showing follicles of various sizes filled with colloid. The trachea is shown at the top of the figure.

to develop, and individuals thus affected are known as "cretins," many being idiots, undersized, and dependent. In some cases, daily administration of thyroxin to infant cretins results in normal development. Myxedema is brought about by atrophy of the gland of an adult, as a result of which there is overproduction of connective and adipose tissue, slowing down of the heart-beat, weakened pulse, thickening of the skin, and dulling of the intellectual powers. Hyperthyroid, on the other hand, produces a condition in which there is a speeding up of basal metabolism, tendency to ingest much food, and an increase in rate of heart-beat. Investigations indicate a functional interconnection between the pituitary and thyroid glands.

17

THE PARATHYROID GLANDS.

In cyclostomes these glands occur as small masses of epithelioid tissue on the ventral portion of each of the seven pairs of gill pouches. In lizards, birds, and mammals they occur as two pairs of small glandular structures. In development, small masses of epithelial cells form as dorsal diverticula from the third and fourth pairs of gill pouches. As the pharyngeal pouches are obliterated by further growth, the four parathyroid anlages separate from their place of origin, migrate backward, and become embedded in the thyroid lobes. (Fig. 159.) Histologically each gland is composed of cords
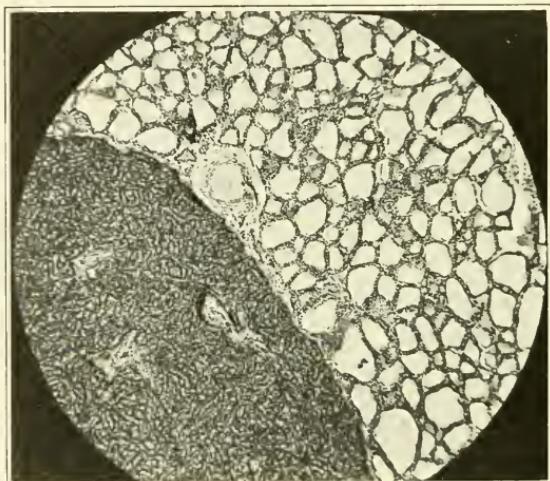
Fig. 159.—Photograph of a section through the thyroid (right) and parathyroid (left) of the dog.

of epithelioid cells, with a capillary network in the connective tissue between them. Two types of cells have been described—chief cells, which are large and pale, and still larger acidophil cells (chromophilic) with granular cytoplasm. The cells are arranged in small masses or columns (Fig. 160) or possibly follicles, in which colloid without iodine collects. In parathyroids of old animals, adipose tissue may develop in the connective tissue between the epithelioid cells. There is no regeneration of parathyroids after their removal.

Removal of portions of the parathyroids causes hyperirritability of the nervous system and sense organs. There is an accompanying decrease in the calcium content of the blood. The normal secretion is essential to normal calcium metabolism, metabolism of sugar,

maintenance of the nitrogen equilibrium, and formation of bone and dentin. Proper combination of parathyroid secretion and calcium salts is essential to normal muscle contraction and administration of parathyroid extract relieves tetany due to faulty function of the parathyroids. Entire removal of parathyroids results in death.

Cords of cells separated by capillaries
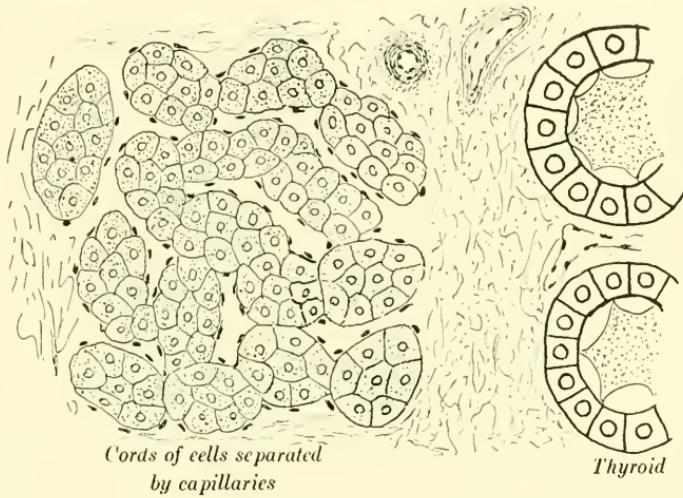
Thyroid

Fig. 160.—Diagram of mammalian parathyroid, showing capillaries between the cords of cells.

THE ADRENAL GLANDS.

In quadruped mammals, one of these glands is located at the anterior end of each kidney. In man they are called suprarenals, because they are located at the upper end of the kidneys. They have no known functional connection with the kidneys. In man they are somewhat flattened, small bodies, the right one being triangular in form, the left somewhat crescent-shaped. In other mammals they are bean-shaped masses, embedded in adipose tissue which tends to form near the kidneys. The adrenals are exceedingly well supplied with blood vessels and it has been estimated that blood to the amount of about five times their weight circulates through them every minute. They are surrounded by a firm capsule of connective tissue, extensions of which pass into the interior of the gland. Cross-sections of fresh adrenal glands made with a sharp knife reveals an outer cortical zone, light yellow in color and of firm texture, surrounding a central medullary region of looser

texture, and dark red in color. The two zones have an entirely different embryonic origin. (Fig. 161.)

The cortex develops as a series of buds from the anterior third of the Wolffian body. The cells of the medulla originate from cells related to those of the cœliac plexus of the sympathetic system and form epithelioid groups. As development proceeds, the latter mass of cells becomes invested with those of the cortical region.

In fishes the medullary cords exist as small masses, each adjacent to a sympathetic ganglion and arranged segmentally alongside the
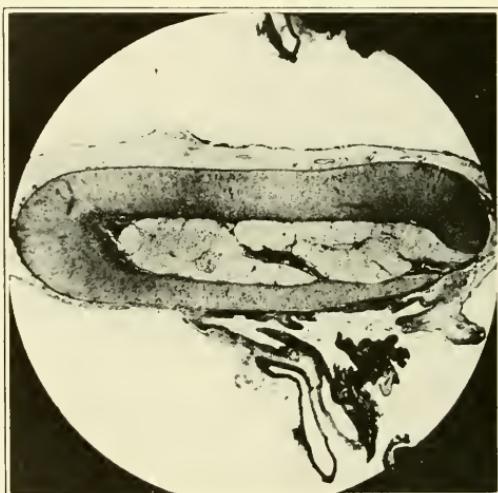
FIG. 161.—Photograph of a longitudinal section through the adrenal of the woodchuck. A connective-tissue sheath surrounds the entire organ. The darker cortex surrounds the central lighter medulla, except at the hilus on the right of the figure. Below are sections of blood vessels.

elongated kidney, while in between the kidneys are two long masses of tissue homologous with the mammalian cortex. In amphibians there is a closer association between the cortex portion and the medulla. In reptiles the two are in contact, in birds they are intermixed, and in mammals the cortex surrounds the medulla.

MAMMALIAN ADRENAL.—Each gland in the case of mammals is surrounded by a connective tissue capsule, which supports arteries, veins, capillaries, and lymphatics. Strands of connective tissue extend in between the epithelioid cells of the cortex into the medulla, supporting a reticular tissue network and capillaries.

The Cortex.—Three zones of epithelioid cells may be distinguished in the cortex. (Fig. 162.) Immediately beneath the capsule is a

narrow zone, the zona glomerulosa, where the polyhedral-shaped cells are arranged in oval groups in the form of flattened or incomplete vesicles. The nuclei stain deeply and the cytoplasm has an affinity for basic dyes. Internal to this is the zona fasciculata, with long double rows of larger polyhedral or somewhat cuboidal-shaped cells, often binucleate, radiating in toward the medulla. In the outer portion of these columns the cells have fat droplets and cholesterol, but usually the technique dissolves the
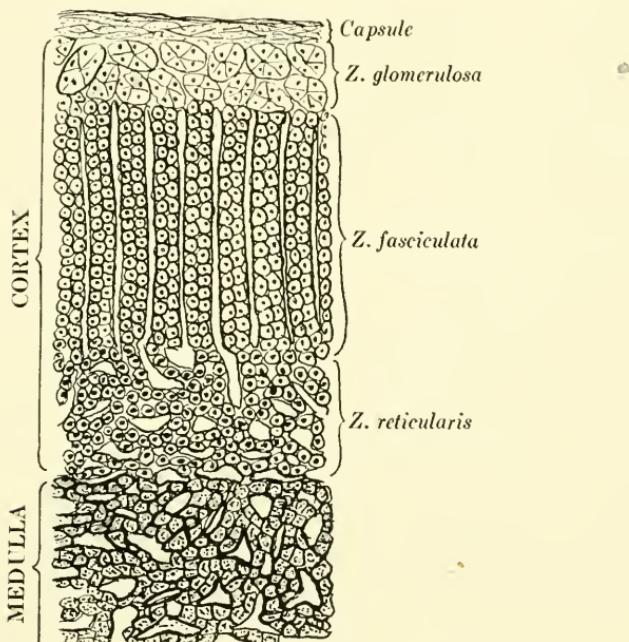
Fig. 162.—Diagram of a section through the adrenal of a mammal.

lipoid substance, so that the cells may have a spongy appearance. Internal to the zona fasciculata is the narrow zona reticularis with cells forming a meshwork of cords one cell in width. These cells contain a brown pigment which increases toward the boundary of this zone with the medulla and becomes more evident in glands of old animals. A capillary net invests the cell groups in the glomerulosa, and from it capillaries extend between double rows of cells of the fasciculata to become a meshwork again in the reticularis, where they connect with capillaries of the medulla. New cells may form by mitosis in the glomerulosa and outer medulla of a mature gland,

and cells degenerating there migrate into the reticularis, where they are removed by macrophages.

The Medulla.—This region consists of an irregularly arranged meshwork of polyhedral cells separated by sinusoidal capillaries. The medulla cells are also roughly rectangular in section, with the longer sides in contact with adjacent cells and with a sinusoidal capillary adjacent to the short sides. When fixed in chromic acid the cytoplasm of the cells is dark brown in color, and if potassium dichromate is used their cytoplasm shows fine brown granules. Because of this affinity for chromium they are referred to as chromaffin cells. In ferric chloride the medulla stains green and in iodine yellow. The fine brown granules are secretory products which reduce dichromate of potassium to chromium dioxide. The number of granules present indicates the degree of secretory activity.

There is a plexus of fine nerve fibers in the cortex, especially in the zona reticularis. Axons of nerve cells are in close relation with the chromaffin cells of the medulla. Here also are a few ganglion cells, or even small ganglia near the central vein. Since the medulla is derived from the same source as the sympathetic nervous system, this central portion of the adrenal is comparable to paraganglia to be described presently. Secretion of the cortical and medullary portions appear to have different functional effects. The cortex is said to produce a special hormone, now called "cortin." Lack of cortin in man, due to pathological lesions of the cortex, results in Addison's disease. Animals deprived of adrenals die, but life is prolonged if extract of cortical tissue is administered.

Epinephrin, or adrenalin, or adrenin, is secreted by cells of the medulla. Circulating in the blood stream, it maintains tone in the small arteries, or arterioles, and so assists in regulating blood-pressure. Local injections of adrenalin cause temporary constriction in small vessels, and in this manner it is used for preventing hemorrhage. Injections of it into a heart that has ceased beating for a short period in some cases results in a resumption of rhythmical contractions.

THE CAROTID GLANDS.

There is a small mass of glandular tissue similar to that of the adrenal medulla at the forking of each carotid artery into its external and internal branch. This mass, known as the carotid gland, has a connective-tissue sheath enclosing a mass of epithelioid cells well supplied with a capillary network. Small sympathetic ganglia are associated with these glands, and the resemblance of the glandular

cells to those of the adrenal medulla suggests an endocrine function, though experimental evidence is lacking.

PARAGANGLIA.

In various organs of the body, occasional sympathetic ganglia are found associated with a small mass of chromaffin cells resembling those of the carotid gland, or adrenal medulla. These glandular masses, known as paraganglia, have a histological structure suggesting an endocrine function, but as yet there is no experimental evidence as to their function.

THE PINEAL BODY.

The pineal body received its name because of its resemblance to a pine cone. It is also known as the epiphysis cerebri, and is located in the dorsal region of the brain between the cerebrum and cerebellum, where it appears as a small red body. It is attached to the third ventricle by a stalk and is surrounded by the pia mater. Islands of epithelioid cells, separated and surrounded by capillaries, are embedded in a connective-tissue stroma. Some of the cells resemble cytons of multipolar nerve cells without any processes. A network of fibers from the sympathetic system pass through the connective tissue. It has been assumed that this glandular tissue has an endocrine function, because it has been observed that a tumorous growth of the pineal gland appears to be associated with an unusually early maturing of the sex glands. That is, it has been assumed that the normal pineal secretes a hormone that delays the too-rapid development of sex organs. However, experiments give conflicting results.

Other Endocrine Glands Already Considered.—The islands of Langerhans in the pancreas secrete insulin controlling metabolism of carbohydrates. The endocrine nature of the ovary and testis has also been indicated. The liver has also had attributed to it an endocrine activity. Possibly all cells have an endocrine activity to some extent, but as yet only the more conspicuous organizations have been demonstrated experimentally.

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See Appendix for general text references.

CHAPTER XVI.

TECHNIQUE.

THE destructive reactions within living cells are counterbalanced by synthesis of elements for repair and continued life. When cells die, syntheses cease and the enzymes involved in vital processes become active in breaking down the products they formerly helped to build. Such disintegration processes working within the dead or dying cell constitute *autolysis*. In addition, bacteria present, or soon entering dead and dying tissues, rapidly add to the disintegration processes and the accompanying distortion or loss of characteristic cellular structure.

To prevent these changes, and also to make cells and intercellular substance insoluble and stainable, tissues from freshly killed animals are immersed in fluids called fixatives. Such fixing fluids should accomplish certain definite things in addition to preventing autolytic and bacterial decomposition. They should penetrate tissues rapidly and coagulate or otherwise preserve protoplasmic substances, leaving intra- and extracellular structures preserved in the same relative spatial relations as during life. They should prevent subsequent changes, such as shrinkage, hardening, and dissolution, and should not hinder later staining. With these requirements for a fixative, let us consider the properties of some chemicals in common use as components of various fixing fluids.

FIXATION.

Fixing Reagents.— *Acetic Acid.*—This precipitates nucleoproteins and, therefore, fixes chromosomes very well, but does not affect the proteins or fats. It causes permanent swelling and distortion of collagenous fibers of connective tissue. Due presumably to its failure to precipitate or otherwise change most of the substance of protoplasm so as to retard its penetration, it diffuses throughout tissues very rapidly. It causes some swelling and leaves the tissue soft.

Picric Acid.—Saturated aqueous solutions of this chemical precipitate all proteins by forming protein picrates, and its pene-

tration is less rapid than acetic acid. It causes some shrinkage but does not harden tissues very noticeably.

Formalin.—Weak solutions (10 per cent) of formalin in water are used as fixing agents. They cause changes in protein by forming additive compounds. The action is slow and the tissues are hardened due to effects on the cell membrane. Shrinkage is not an immediate result but may follow during later stages in technique.

Alcohol.—This precipitates proteins, the precipitates of nucleo-proteins being soluble in water. It dissolves lipoids but precipitates glycogen. Tissues treated with alcohol shrink and harden and later staining is difficult.

Osmium Tetroxide.—Solutions of this chemical in water are called osmic acid. This fixes and blackens fat, chondriosomes, and the Golgi apparatus. Its effect upon the remainder of the proto-plasm is one of fixation without precipitation. Shrinkage is slight, penetration is slow and variable, and staining is made difficult.

Chromic Acid.—Solutions of this reagent act as oxidizing agents. It precipitates proteins presumably by forming compounds with them, but leaves fat and lipoids unaffected. It should be washed out with water, so that interfering precipitates are not formed, as they would be if washed with alcohol. The chromic acid solution causes slight shrinkage and hardening but makes staining with basic dyes easier.

Potassium Dichromate.—Unacidified solutions of this reagent render albumin insoluble but do not precipitate it. It is similar to osmium tetroxide in that it renders proteins insoluble without precipitating them. Chromatin is dissolved, so that it is a poor chromatin fixative. Its action is slow, and tissues should be washed in water after its use. When made acidic, its solutions fix in the manner of chromic acid.

Mercuric Chloride.—Saturated solutions of this reagent in water act as precipitants of proteins, have no effect on lipoids, and do not destroy chondriosomes. The solution penetrates quickly and causes some shrinkage. It is necessary to wash it out after short fixation. Crystals tend to form and should be dissolved later by washing in alcohol to which iodine has been added.

These are but a few of the numerous reagents in use, and only a brief note is made of their action. For example, it was just stated that acetic acid and picric acid are both useful, but that the first causes swelling of tissue and the second causes shrinkage. By a proper combination these effects may be counterbalanced. A review

of the actions of the reagents noted above indicates a reason for the protoplasmic conditions observed in fixed tissues. We will now indicate a few fixing fluids found useful in general work.

Fixing Fluids.—*Bouin's Fluid.*—The formula is as follows: 75 parts saturated aqueous solution of picric acid; 25 parts formalin (40 per cent formaldehyde); 5 parts glacial acetic acid. This is one of the best general fixatives, and in it the effects of the three chemicals are well balanced to give a fair preservation of cellular structure. Chromosomes are especially well fixed. Glycogen, fat, chondriosomes, and the Golgi apparatus are not usually preserved. Tissues may be left for long periods in it without harm, but twelve to twenty-four hours is ordinarily an adequate time. There is little or no hardening or shrinkage, and tissues will later stain well. After fixation the tissue may be washed in 50 or 70 per cent alcohol to get rid of the free fixative remaining.

Flemming's Fluid.—The formula is 1 per cent aqueous solution of chromic acid, 15 parts; 2 per cent aqueous solution of osmium tetroxide, 4 parts; glacial acetic acid, 1 cc., or even less, to be added just before using the fixative. This solution is useful for fixing chromosomes, chondriosomes, and fat. The amount of acetic acid should be reduced to a few drops for better results with chondriosomes. The osmic acid fixes the fats and chondriosomes. The acetic acid fixes the chromosomes and aids in preventing shrinkage. The chromic acid fixes proteins and chromosomes. However, fixation may be uneven, part of the tissue being overfixed, part properly fixed, and the inner portion of the tissue being underfixed. Fixation should extend over a twenty-four-hour period when acetic acid is present to the extent of 1 part, but the period should be extended to four days when the acetic acid is reduced and when it is desired to retain chondriosomes. After fixation the tissues should be washed for twelve hours in running water to remove all the free fixative present before going ahead with the technique.

Zenker's Fluid.—The formula is 2 grams potassium dichromate; 1 gram sodium sulphate; 5 grams mercuric chloride; 100 cc. water; 5 cc. glacial acetic acid. The acetic acid should be added just before using the fluid. Tissue should be fixed for twelve hours. Long fixation causes formation of crystals in the tissue. After fixation the tissues should be washed in running water for several hours, then transferred into 70 per cent alcohol to which iodine has been added. The latter becomes clear, due to the extraction of mercury salts from the tissue. Fresh iodized alcohol should be

used again until the retention of the iodine color indicates that all free mercury salt has been removed from the tissues. The tissue then may be stored in 70 per cent alcohol.

A modification of Zenker's fluid is called *Helly's fluid.* It has the same formula as the above, but substitutes 5 cc. of formalin for the acetic acid. It gives an excellent picture of protoplasmic structure, but care must be taken in later steps in technique so that distortion through shrinkage does not take place. Chromosomes are not so distinct as with Zenker's fluid, but cytoplasmic structures are well preserved and can be demonstrated by various stains. Helly's fluid must be thoroughly washed out in running water after twelve hours' fixation, and the tissues must also be later immersed in 70 per cent alcohol to which iodine has been added.

Formalin Solutions.—A 10 per cent solution serves as a good preservative and fixative in the case of delicate tissues that will stand hardening. It has been used as a preliminary reagent in a number of techniques employed for nervous tissue. Tissues fixed in it should be transferred to 70 per cent alcohol after twelve hours' treatment with the formalin solution. Shrinkage often occurs later during paraffin embedding.

Carnoy's Fluid.—The formula is: 75 cc. of 100 per cent alcohol and 25 cc. of glacial acetic acid. This fixing fluid fixes chromosomes well, precipitates glycogen, but usually dissolves chondriosomes and the Golgi apparatus. It penetrates quickly, so that an hour serves to fix soft pieces of 1 cm. in thickness. It combines the effects of two chemicals, the alcohol causing precipitation of the proteins and glycogen of the cytoplasm; the acetic acid fixing the nucleoproteins and preventing some of the shrinkage and hardening of the alcohol. Tissues should be washed in absolute alcohol after fixation before proceeding to embed.

For additional information concerning fixatives the student is referred to the texts cited at the end of this chapter, but for a general treatment of fixation reactions it is recommended that J. R. Baker's monograph on "Cytological Technique" be studied.

Containers.—As containers for the fixative and excised material, it will be found advantageous to use wide-mouthed bottles of 1- or 2-ounce capacity provided with cork stoppers. In order that the fixative may reach all parts of the surface of the material, it is well to place a small bit of absorbent cotton in the bottom of each bottle to prevent the tissue from adhering to the glass, and so keeping one surface from free contact with the fixing fluid. The bottle

should be well filled with the fixative so that there will be many times the volume of the tissue to be fixed. The small amount of water from the tissues should not dilute to any appreciable degree the concentration of the fixing fluid.

HANDLING THE MATERIAL.

Dissection. — Pieces of tissue should be small. This is important for good fixation. Generally pieces, 0.5 cm. to 1 cm. in thickness, can be used. The length and breadth of the piece should likewise be kept to the smallest practical size. The pieces should be handled as little as possible with dissecting instruments and care should be taken not to compress them during dissection. The operator should keep in mind that he is later going to make sections of these pieces, and should remove material from the desired portion of the organ in the proper manner for longitudinal, cross-, or tangential sections. The selected pieces can be transferred from the animal to the bottles of fixing fluid with a spatula, or lifted lightly with forceps, without permitting the instruments to enter the fluid. If dissection is prolonged, it is advisable to keep the organs of the freshly killed animal moistened with the proper physiological saline solution (0.85 per cent sodium chloride for mammals; 0.7 per cent for amphibians).

Foreign Matter. — In fixing parts of the alimentary canal, it is necessary to remove the contents of the lumen. This may be done by washing out with physiological saline before cutting out pieces for the fixing fluid. Or fixing fluid may be forced under gentle pressure into the lumen of the canal with a pipette. If the bladder is dilated with urine, the neck of the bladder can be ligated, and then the entire bladder removed and placed in a beaker of fixing fluid for a few minutes to stiffen the wall in the extended position. The bladder then can be opened, emptied, and so cut that fixative can freely come in contact with the internal surface. The stomach can be handled in the same fashion. Or a small strip of stomach wall can be placed on a strip of stiff paper and thus immersed in the fixative for a few minutes, and then, when stiffened in the extended condition, it can be removed from the paper and entirely immersed in fixative. Pieces of artery or nerve can likewise be placed extended on a paper strip, and this placed in fixative until the pieces are stiff enough to be removed from the paper for further fixation.

Heat. — Fixatives are ordinarily used at room temperatures, but the effect is hastened by increasing the temperature. Heat itself

is a coagulant of protoplasm, and its effect must be considered in
addition to the action of the fixative itself. It will be found that
the final picture obtained by different temperatures of the same
fixative and the same material will show certain differences, and
these modifications should be checked carefully.

Records.— One of the most important things to remember is to
keep a careful record of the material, the organ or part of the organ
removed, the age and sex of the animal, the fixative used, the time
of fixation, and any modifying conditions, such as health of the
animal and method of killing.

These details may be kept in a note-book and corresponding code
numbers placed with the fixed material, or the entire information
may be recorded on the slip that accompanies the fixed material.
The writing on these slips should be done with a soft pencil. To
avoid confusion, do not place many tissues together in a bottle.
It is wiser to place not more than three small pieces in each bottle
so that no difficulty later arises in identifying them for further
steps in the technique.

PARAFFIN EMBEDDING.

We have indicated the proper length of time needed for fixation
with each of the fluids described above. It has also been shown
that the fixed tissue should be thoroughly washed free of all fluid
fixative remaining. Usually the tissue pieces are preserved in
70 per cent (or 80 per cent) alcohol. The next problem is to prepare
the material for cutting sections which after proper staining can
be studied microscopically for their structural organization. Prior
to the development of modern technical processes, the early workers
at first held pieces of tissue or organ in one hand and cut thin slices
of it free hand with a sharp knife or razor. However, a piece of
fresh or fixed tissue "gives" when sections are cut in this manner,
and the material is more or less crushed. This is avoided if the
tissue is infiltrated with paraffin. Paraffin is solid at room tem-
peratures, but a simple yet somewhat time-consuming technique
enables one to satisfactorily impregnate the tissue piece with paraffin.
This is accomplished as follows:

Paraffin is not miscible with water, so that all water must be
removed from the tissues. To accomplish this, the 70 per cent
alcohol in which the piece of tissue has been kept is discarded and
80 per cent alcohol replaces it. After an hour or so, the 80 per
cent is discarded and replaced with 95 per cent alcohol. An hour

or so later this is changed to 100 per cent alcohol. This has no water in it, and when diffused through the tissue, water will have been removed. This process of dehydration must not be too rapid or distortion, caused by too rapid withdrawal of water, may occur. The alcohol may also exert an effect on some of the protoplasmic substances which have been preserved but not precipitated or coagulated by the fixatives. Tissues should not remain too long in the higher alcohols, *i. e.*, 95 and 100 per cent.

Paraffin does not mix with alcohol; therefore, some other common chemical must be employed that is miscible with alcohol and paraffin which can be used to transfer the object from alcohol to paraffin. Xylol or benzene can be used for this purpose. Xylol is preferable, since it is not so inflammable as benzene, although benzene causes less shrinkage than xylol. Therefore, transfer the tissue from 100 per cent alcohol to a mixture of equal parts of 100 per cent and xylol for a certain period (one hour); then change to pure xylol for one to two hours; then pure xylol and paraffin for a similar period. If the paraffin used has a melting-point of 50° to 52° C., then the mixture of xylol and paraffin must be kept at a temperature not far below this to keep the mixture in solution and so enable it to diffuse all through the tissue. It should be remembered that there must be no trace of water left in the tissue. So far it is infiltrated with a mixture of one-half xylol and one-half paraffin.

This mixture is discarded and the bottle is filled with pure melted paraffin at a temperature just above its melting-point, *i. e.*, 55° C. In order to maintain the paraffin in a liquid condition, the supply of melted paraffin and the specimen bottles containing tissues being embedded are kept in a constant temperature oven, sometimes called a paraffin oven. After a certain length of time (one hour) the first supply of paraffin is discarded and replaced with fresh paraffin and placed back in the oven for another hour. It is essential to remove all traces of xylol and to have the pure paraffin diffuse all through the tissue. It is even advisable to change the paraffin once more for another hour in the oven.

A paper boat or a glass dish (Syracuse dish) coated with glycerin is now filled with pure melted paraffin, and the tissue piece poured with its paraffin or transferred to this dish with warmed forceps or spatula. The tissue piece is quickly and properly oriented with a view to the type of sections to be made later. Then a surface film of solid paraffin is produced by blowing across the top of the dish, and the container is gently immersed in cold water for a time, until

the paraffin hardens throughout homogeneously and quickly. In a few minutes the hardened paraffin block can be removed and stored indefinitely in this form or prepared for sectioning. If the weather is very warm, or if the technique is carried out in a warm room, one would use a higher melting-point paraffin, while a lower melting-point paraffin is suitable for use in winter or under colder conditions. If very thin sections are required, one must use a harder (higher melting-point) paraffin than if thicker sections were to be made. Ordinarily it is wise to avoid heating the tissue any higher than absolutely necessary in order to avoid the hardening and shrinking effect of heat, so that 58° C. represents a maximum temperature for the paraffin bath.

In the foregoing account of dehydration we passed from 95 per cent alcohol to 100 per cent alcohol and from this to xylol. Since 100 per cent alcohol is expensive, and since it tends to harden tissues, it can be dispensed with by the use of aniline oil. This is cheaper and does not harden or shrink the tissues. The following tables explain the two procedures: It is understood that the tissue has been properly fixed, washed free of fixative, and has been stored in 70 per cent alcohol.

<center>Procedure.</center>

Absolute Alcohol Method.	*Aniline Oil Method.*
80 per cent alcohol, one hour.	$\frac{2}{3}$—80 per cent + $\frac{1}{3}$ aniline, one hour.
95 per cent alcohol, one hour.	$\frac{1}{3}$—95 per cent + $\frac{2}{3}$ aniline oil, one hour.
100 per cent alcohol, one hour.	Aniline oil (until translucent), one hour.
$\frac{1}{2}$—100 per cent + $\frac{1}{2}$ xylol, one hour.	$\frac{1}{2}$ aniline + $\frac{1}{2}$ xylol, one hour.
Xylol, one hour.	Xylol, one hour.
Fresh xylol, one hour.	Fresh xylol, one hour.

$\frac{1}{2}$ xylol + $\frac{1}{2}$ melted paraffin, one hour and kept in warm place so that the paraffin remains melted.
Pure melted paraffin (about 52° C. m.p.) in the paraffin oven, one hour.
Change the paraffin and keep specimen in oven, one hour.
Change the paraffin again and keep specimen in oven for about one hour.
Embed as directed above.

> Toluol is an excellent substitute for xylol and tissues can be left in it for longer periods. Furthermore, it is more volatile and therefore more readily lost during paraffin transfers.

CELLOIDIN EMBEDDING.

For material which is tougher, or which demands a technique which does not involve heating, a slower method of embedding in celloidin instead of paraffin has been devised. It has the advantage of causing less shrinkage and distortion but does not permit such thin sections to be easily cut. Only single sections are made at a time, and so the handling of large numbers of sections is more

time-consuming than is the case with paraffin sections. Solutions of celloidin in an ether-alcohol mixture are utilized in celloidin infiltration.

Celloidin is furnished in small solid pieces. Remove all the pieces from a 1-ounce bottle, dry them thoroughly, and place in a glass-stoppered bottle, adding about 150 cc. of absolute ethyl alcohol and 150 cc. of sulphuric ether. Stopper the bottle and keep the stopper free of the solution at all times. It will take several days before the celloidin has completely dissolved. This will make a stock solution of concentrated celloidin, which we will call the No. 1 solution. When it is in complete solution, make stock solution No. 2 by diluting part of No. 1 with about three times as much of equal parts of absolute alcohol and ether. Also make stock solution No. 3 by diluting solution No. 1 with about ten times as much of equal parts of absolute alcohol and ether. Keep a supply of each of these three solutions on hand, taking care that each is kept in a tightly stoppered bottle, as evaporation of the ether-alcohol soon changes a thin solution to a thicker one.

Tissue in Bouin's or Zenker's fluid can be embedded in celloidin as well as those especially designed for nerve study. It is well to use thin pieces, *i. e.*, about 0.3 cm. thick, because infiltration proceeds slowly and with difficulty. Each successive fluid presently named must penetrate the tissue piece, and as the size is increased the time needed must be extended. It is suggested that the student at first use a piece of soft tissue, such as the liver, about 0.5 by 0.5 by 0.3 cm., and follow the longer time period given in the following table. We will suppose that the tissue has been fixed in Bouin's fluid, washed, and taken from 70 per cent alcohol, where it has been preserved. The process takes place at room temperature and in bottles that can be tightly stoppered. The time can be shortened by keeping tightly covered bottles at a temperature of 40° C.

CELLOIDIN EMBEDDING METHOD.

From 70 per cent alcohol to 95 per cent, for two hours.

From 95 per cent to fresh 95 per cent alcohol, for one hour.

From 95 per cent alcohol to 100 per cent alcohol, for two hours.

From 100 per cent alcohol to fresh 100 per cent, for twelve to twenty-four hours.

From 100 per cent alcohol to one-half absolute alcohol + one-half ether, for twelve to twenty-four hours.

18

From ether-alcohol mixture to celloidin No. 3 (thin), for three
to four days.

From celloidin No. 3 to No. 2 celloidin (medium), for four to
six days.

From celloidin No. 2 to No. 1 celloidin (thick), for five to eight
days.

At the end of this time the tissue should be completely saturated
with celloidin. We can proceed from this point in two ways:

Fashion a little paper receptacle and fill it with celloidin No. 1.
Add but a few drops at first and wait a moment until they thicken,
add a few more drops, and then fill the receptacle. The tissue piece
is then placed in the box and arranged properly, with a view to
plane of sections to be cut later. The surface of the celloidin
rapidly forms a film when exposed to the air. Evaporation of the
solvent (ether-alcohol), with consequent hardening, should be per-
mitted to proceed slowly until the celloidin mass is like a heavy
gum in consistency. The paper receptacle is now carefully trans-
ferred to a covered glass dish containing chloroform, where hardening
is completed. The chloroform causes the celloidin to harden suffi-
ciently in one to two hours, when the paper box and excess celloidin
can be cut away and the embedded block can be placed in a bottle of
one-half 95 per cent alcohol + one-half glycerin until ready for
cutting.

The other method both embeds and mounts the tissue preparatory
to sectioning. Vulcanized fiber blocks formed to fit the clamp of
the microtome should be used. Do not use wood blocks. The
solvent in the celloidin mixture extracts oils from wood and these
discolor the preparation. A strip of stiff paper is fastened about
one end of the fiber block so that a receptacle is formed at this end.
Celloidin No. 1 is added, drop by drop, to this receptacle, covering
the bottom of it with a relatively solid coat which is allowed to
thicken before filling the receptacle with celloidin from the specimen
bottle. The tissue is transferred also and oriented in the celloidin
which covers it on all sides. The celloidin is then permitted to
harden somewhat in the air, as in the first case, and then the block
with the celloidin-filled receptacle is transferred to a covered dish
of chloroform. After hardening in chloroform the block is stored,
as in the first procedure.

If the first method was used it is necessary to fasten the celloidin
block to a fiber block before cutting sections. To do this thick
celloidin (No. 1) is poured over one end of the fiber block and over

one end (bottom) of the celloidin block. The two are pressed together; the celloidin at the junction is hardened in the air until it is thick, then in the chloroform as before.

SECTIONING.

As soon as material is properly embedded one may proceed directly to cutting sections for staining. Machines, called microtomes (from the Greek, "to cut small"), have been devised and can be regulated to cut sections of desired thickness easily and uniformly. Two general types of microtomes are in common use: one, working on a rotary principle, is called a rotary microtome (Fig. 163), and the other, cutting by sliding movements, is known as the sliding microtome. The rotary type is suited for cutting
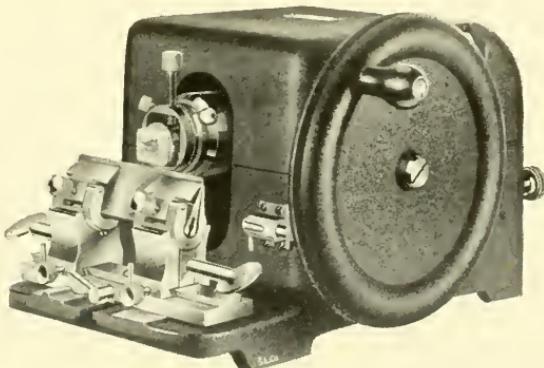
Fig. 163.—Illustration of the rotary microtome. (Courtesy of Spencer Lens Co.)

paraffin embedded material, while celloidin material is sectioned with the sliding microtome, although the latter may be used for paraffin material also.

Rotary Microtomes and Paraffin Sections.—The illustration presents the principal features of this type of microtome. The following information should be supplemented by classroom demonstration in the use of the machine and also by considerable actual experience in section cutting by each student. Small metal discs or wooden blocks are furnished to which the paraffin block may be attached. These supports can be then fastened into a holding device in the microtome.

We will suppose that we have a disc of paraffin containing three pieces of embedded tissue. One of these is cut out of the disc by

means of a warm scalpel blade, leaving plenty of paraffin around
the tissue. To fasten this paraffin block to metal disc or wood
block, an old scalpel blade is heated so that it will easily melt
paraffin. The blade is then held against the metal disc or paraffined
end of a wood block, and then the paraffin piece is pressed against
the other surface of the blade. The blade is withdrawn and the
melted paraffin thus produced hardens and seals the paraffin block
to the metal or wood holder. It is advisable to place mounted
tissue in cold water for a minute or two to completely harden the
paraffin.

The next step is to trim the paraffin block containing the tissue.
Some of the extra paraffin about the object is cut away, so that the
piece is in the shape of a truncated
pyramid, with the base toward the
wood block or metal. The paraffin
at the free end is trimmed down until
little is left between the end face and
the tissue. The free face should be
rectangular or square in form, and
the angles between adjacent edges
should be right angles. (Fig. 164.)

Fig. 164.—Diagram illustrat-
ing paraffin block ready for sec-
tioning.

It is an easy matter to prepare a
considerable number of small wood
blocks, and these can be furnished to
all the students who can proceed to
mount embedded material and have it
ready for sectioning. Or if one has a
good many paraffin blocks to section,
it is more efficient to mount all on
wood blocks at one time and store
them in boxes properly labelled for
sectioning later. Storing paraffin blocks in water a week or more
before cutting makes cutting easier and prevents brittleness and
cracking of the tissue.

The clamp holding the mounted paraffin-wood block is attached
to a movable part of the microtome that is connected with a finely
threaded screw. The turn of the wheel turns the screw and ad-
vances the part holding the paraffin block. It will be noted that
in this process the paraffin block rises and falls. Each machine
has a setting device by which the thickness of the sections can be
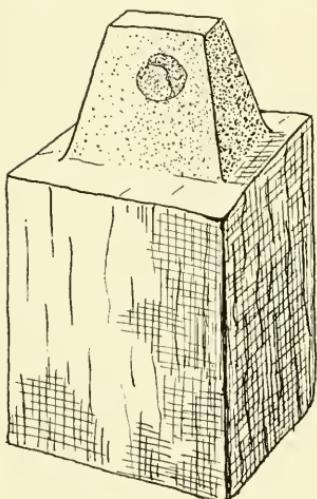
arranged. The setting device has a rather wide range of adjustment. Sections 10 microns thick are made in general work in microscopic anatomy. The special microtome knife is held in a clamp that can be moved by hand, but must be locked rigidly in place while sectioning. Suppose the setting device is adjusted for sections 10 microns thick, and the wheel is turned. Between the time that the paraffin block passes above the knife edge and then descends to it on the down stroke, the paraffin block has advanced forward exactly 10 microns, but does not advance after the tissue passes below the knife edge. So a section 10 microns thick is cut from the anterior face of the paraffin block each time on the down stroke. However, the position of the paraffin block must be correct. The trimmed end should have the form of a rectangle or square. When the knife is placed in the knife-holder, the back face of the knife should make a slightly acute angle with the plane of the table. The lower edge of the face of the paraffin block should be exactly parallel with the edge of the knife. The knife-holder should be advanced so that the edge of the knife is nearly in contact with the descending block of tissue and then locked. The wheel of the machine is then turned and sections begin to be cut. If conditions are correct, each section will remain with its upper edge just along the front cutting edge of the knife. When the next section is cut, its lower edge will fuse with the upper edge of the previous section because the friction of cutting melts the paraffin along this line. Thus a ribbon of sections is formed. One *could* cut a ribbon many feet in length. However, it is more convenient to make one about 10 inches in length and then transfer this to a shallow paper box. To do this, the outer end is supported with a section-lifter held in the left hand while cutting. When the desired length is cut, the end next to the knife is carefully lifted off with a scalpel or section-lifter and the length of ribbon placed carefully in the paper box. When the next ribbon is cut, it is laid alongside the first in the box and so on, until the desired number of sections are obtained. If one desires to study all parts of an organ, such as the submaxillary gland, it is necessary to save all the ribbon sections and all the sections can be mounted in regular order, thus making a complete series of slides covering the entire microanatomy of that organ. Ribbons of sections stored away in shallow drawers or paper boxes can be kept indefinitely provided they are stored in a cool, dust-proof place. If kept in a warm room or one which becomes very warm on hot summer days, there is a tendency for the thin paraffin

to melt, and the ribbons may become attached to the bottom of the receptacle in which they are stored.

The Sliding Microtome and Celloidin Sections. —The accompanying illustration presents an idea of a present-day example of the sliding microtome. (Fig. 165.) The knife is mounted in a holder that fits over the smooth surface of the sliding device. The position of the knife may be fixed by the screws that clamp down on the handle. The fiber block with the attached celloidin block containing the embedded tissue is fastened into the clamp at one
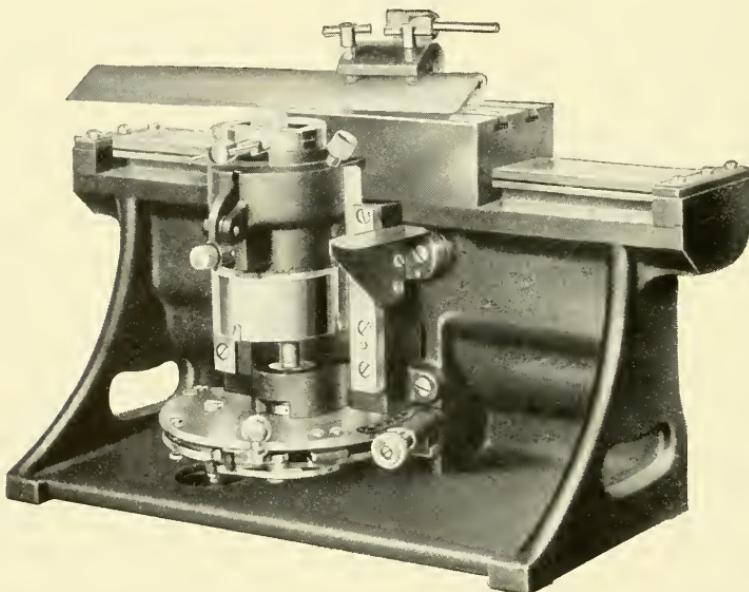
Fig. 165.—Illustration of a sliding microtome. (Courtesy of Spencer Lens Co.)

side of the slide over which the knife is carried. The clamp holding the embedded block is connected with a device which is essentially a fine-threaded screw to which a micrometer is attached. When the micrometer disc is properly turned a certain distance, the screw lifts up the embedded block a certain definite distance above the cutting edge of the knife. The knife must be arranged at an oblique angle with the line of the slide carriage. Both knife and embedded block are kept moistened with 70 per cent alcohol through the sectioning process. The knife is drawn past the celloidin block with the right hand. One can determine by means of the micrometer disc how far to turn the screw, and so determine the thickness

of the sections to be cut. The strokes of the knife should be smooth and rapid. The knife should have been correctly sharpened to secure good sections. Sections of from 10 to 30 microns are usually cut, but with practice thinner ones may be obtained. Single sections are obtained by this method, and these must be transferred with a camel's-hair brush as they are cut to a dish containing 70 per cent alcohol.

Mounting Paraffin Sections.—After paraffin sections have been cut and transferred to a paper box, the next step is to attach them to slides. The sections as they come from the microtome are usually slightly compressed or wrinkled, due to the pressure as they cross the knife. This must be remedied when the sections are attached to the slides. In the first place the slides must be thoroughly clean so that no dirt or grease particles are on their surfaces. They can be cleaned by boiling in soap and water, thoroughly rinsed in water, then rinsed in 95 per cent alcohol, and dried with a clean cloth free from lint or dust. Next, the sections must be made to adhere to the slide to permit the subsequent steps of the staining technique. A solution of equal parts of carefully strained egg-white and glycerin is used as an adhesive. A small amount of thymol or methyl salicylate added to the adhesive mixture prevents disintegration by bacterial action. A common method of applying the egg-albumen adhesive is as follows: A small drop about the size of the head of a pin is placed in the center of one surface of the slide, and this drop is carefully smeared over the surface with the ball of the little finger that has been thoroughly washed. A drop or two of distilled water is then added to the surface. Then a small piece of ribbon containing one or two sections of the tissue is cut from the long ribbon with a scalpel and transferred to the water over the egg albumen on the slide. It is advisable to use water that has been boiled (and cooled) to avoid bubbles gathering between the slide and the sections. The ribbon piece should be arranged parallel with the length of the slide. Two or even three small ribbon pieces may be arranged alongside each other. The same surface that is upward when the ribbon comes from the microtome should be upward on the slide. The under side is more shiny than the upper. After the ribbon pieces have been arranged, the slide can then be transferred to a warming table (Fig. 166), the surface of which is kept at a temperature of about 45° C. or a little less. More water can be added to float the sections but not enough to run off the slide. The gentle heat warms the

paraffin enough so that the surface tension of the water will pull the sections out flat. Sometimes sections are badly folded, and in that case one can carefully pull them out flat when the slides are on the warming table. However, such sections are never as satisfactory as those which are comparatively flat when received from the microtome. The slides can be left on the warming table until all the water has evaporated or the excess water carefully removed after the tissue is expanded. Care should be taken that no air bubbles are left under the sections, since the sections are free from the glass at such points and may later fall off the slides or make
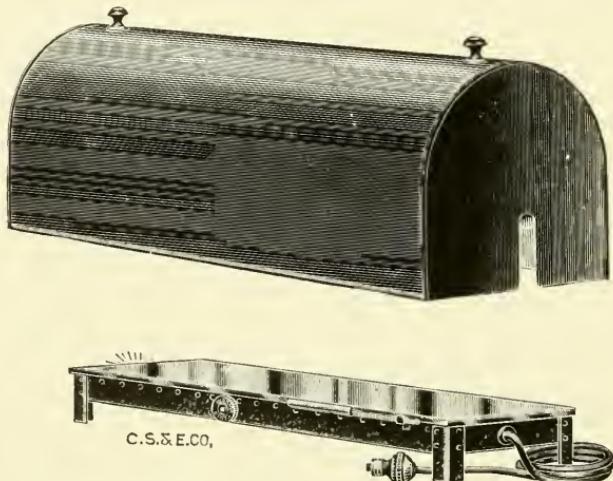
Fig. 166.—Illustration of a warming table. (Courtesy of E. Leitz & Co.)

uneven places in the stained and mounted sections. The heat used in flattening the section should not be great enough to liquefy the paraffin of the ribbon pieces.

A second scheme for fastening sections to slides is to make a solution of 1 part of egg-albumen fixative to 100 parts of cool boiled water. This thin adhesive is added directly to a clean slide, with a pipette, and the ribbon pieces floated on it; the remainder of the process is similar to the foregoing. A third method is to fill a large shallow vessel with water which has been warmed to about 45° C. Small pieces of ribbon are floated on the water. It will be noted that they flatten out immediately. Then a clean slide smeared with egg-albumen fixative can be lowered under such a ribbon piece to remove it. After draining away the excess water and arranging the section, the slide can be set aside until all the water

has evaporated. Good results are obtained from badly wrinkled sections by this method.

In all three cases, the slides should be set aside in a dry place until all the water has evaporated before staining; usually this should be about twenty-four hours.
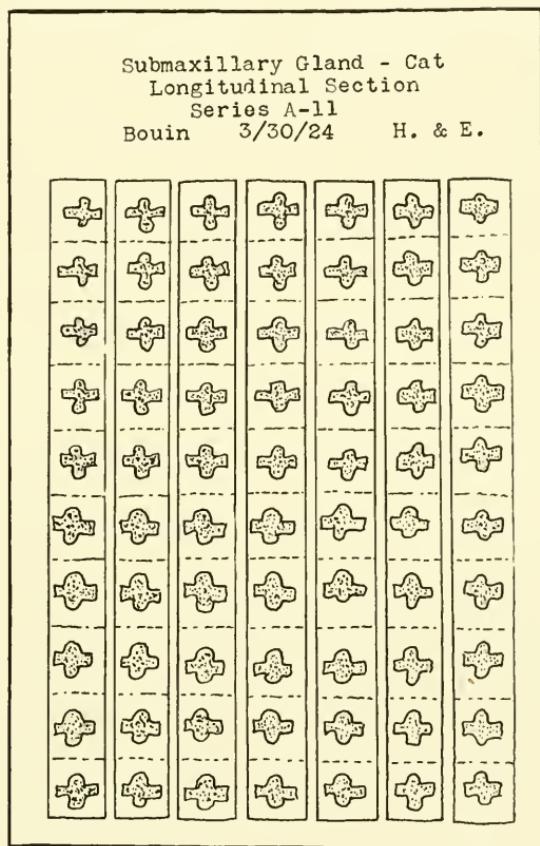
Fig. 167.—Diagram of a preparation of serial sections.

Serial Sections.—After becoming proficient in cutting sections, the student may wish to cut an entire organ into sections and mount these in order. When finally prepared one can study the micro-anatomy of the organ from one end to the other. Usually trans-verse or longitudinal sections are made. If there is a right and left gland, cross-sections can be made from one and longitudinal from the other. The attempt should be given up if the tissue does not section well. We will suppose that it has been possible to make

complete ribbon cross-sections of the entire organ, and these have been placed in order in the paper boxes.

If many sections are to be mounted, the student can use larger slides. After the slide is labelled and the albumen added, then, beginning at the front end of the series of sections, one removes a strip *less* than 2 inches ($1\frac{1}{2}$ inches) in length and carefully places this along the edge of the upper surface of the slide, as indicated in the diagram. (Fig. 167). Then adjacent to this, the next strip, and so on. Leave an empty space, about 0.5 cm., along each lateral margin. Place the slide on the warming table, add water, orient, and dry as in single mounts. The number of sections one can get on a slide depends on the size of each section. After completing the mounting of Slide 1, proceed with 2 and remember to label it. Proceed until all the sections are mounted.

STAINING OF PARAFFIN SECTIONS.

Thin sections of fresh living material examined with the aid of a microscope appear homogeneous, since there is little optical differentiation of cellular structures. Similar examination of fixed tissues which have been cleared reveal more details of structure and organization. These are brought out most distinctly and definitely, however, by the proper treatment of the sections with various dye solutions. The color of the dye or stain is adsorbed, absorbed, or chemically held by the different elements of the cell protoplasm in a characteristic manner. Early studies revealed the fact that the nucleus is acid in reaction and has an affinity for basic dyes, while the cytoplasm is basic and has an affinity for acid dyes. When two dyes, one acid and one basic, are used on tissues, the nuclei and the cytoplasm are differently colored, so that a differential staining effect is obtained. Differentiation of various protoplasmic elements has been the aim of numerous staining techniques, so that it is now possible to employ special processes to demonstrate such cellular structures as centrioles, cell membranes, chondriosomes, Golgi apparatus, secretion granules, fat droplets, nucleoli, chromosomes, chromidia, glycogen, elastic fibers, Nissl bodies, and many others. These special techniques can be experimented with after the student has become familiar with the more common general methods used to demonstrate tissue organizations.

For this purpose we will describe in some detail a few general techniques.

Progressive Staining. In progressive staining the object is to treat the sections with the stains until the tissue has the proper

color. This is determined by observing the progress of the staining under the microscope at short intervals during the procedure. It is not advisable to stain too deeply, since later removal is never altogether satisfactory, although it is possible. In the following method we will employ Harris's hematoxylin as a nuclear stain and eosin as a cytoplasmic stain.

After preparing the nuclear and cytoplasmic stains, it is necessary to assemble a series of jars just large enough to hold the slides and in sufficient numbers to contain all the reagents needed. Shell vials, a little over 1 inch in diameter and about 4 inches long, are useful for the beginner. The Coplin jar is a more useful vessel, since it can accommodate at least five slides at one time. Fourteen of these jars will be found adequate. The fluid in each should extend about 2 inches from the bottom, so that the upper third of the slide will be free of fluid.

Following is a list of jars and the fluid in each: (1) Xylol; (2) aniline oil; (3) 95 per cent alcohol; (4) 70 per cent alcohol; (5) 50 per cent alcohol; (6) distilled or tap water; (7) hematoxylin stain (one-half stock solution, one-half water); (8) water; (9) 50 per cent alcohol; (10) 70 per cent alcohol; (11) eosin in 70 per cent alcohol; (12) 95 per cent alcohol; (13) aniline oil; (14) xylol.

When using paraffin sections the paraffin must be removed before proceeding, since paraffin does not mix with either alcohol or water, and the dyes are dissolved in the latter. The slide is placed in the xylol jar for about three minutes, during which the paraffin will dissolve. After this step the sections should never be permitted to remain out of the fluids until permanently prepared. After the xylol, the slide is drained of free xylol and placed in the aniline oil jar for two minutes, then drained and placed in the 95 per cent alcohol for two minutes, and similarly in the 70 per cent, 50 per cent, and water for two minutes each.

The slide is placed in the hematoxylin stain for three to five minutes, then rinsed in water and examined under the microscope. The section should have a rich purple or blue appearance. Microscopic examination should show a purplish chromatin network in the nuclei of the preparation. If the general appearance is a light lavender, the section is understained. It should then be placed in the stain for a moment or so more and reëxamined. If the section appears dark purple it is probably overstained. In progressive staining it is desirable to stain until just the right effect has been produced and then to stop further staining by placing the section

in water. If overstained, the excess can be extracted by placing the slide in 70 per cent alcohol to which a drop or so of hydrochloric acid has been added. However, this is not satisfactory with this type of stain. We will suppose that the correct staining effect has been attained without extraction. After rinsing in running tap water for fifteen minutes the slide is placed in 50 per cent alcohol for a minute or so, then in 70 per cent alcohol for two minutes. It is next placed in the cytoplasmic stain, such as 70 per cent alcohol and eosin, for thirty to fifty seconds. It should be removed and rinsed in 95 per cent alcohol and examined for the cytoplasmic stain effect which should not be too deep in color. Then the slide is placed in aniline oil for two minutes, drained, and placed in xylol for two minutes, and placed in the first xylol jar for two minutes more. The purpose of the two xylol baths is to prevent carrying over any aniline which would stain the final preparation. The student should have at hand a bottle of Canada balsam, or gum damar, dissolved in xylol to a relatively thick viscous consistency but thin enough to flow easily. Clean and thoroughly dry cover-glasses should also be ready. A pair of fine forceps will also be needed. The slide is then taken from the second xylol jar, the undersurface wiped dry, placed on filter paper on the table and 1 or 2 drops of the balsam placed over the section. A cover-glass is lifted with the forceps and carefully lowered over the section. It is well to let one edge of the cover-glass rest at the side of the slide and then to let it fall gently by its own weight over the section, thus preventing air bubbles from collecting under the cover. After the section has thus been covered, it should be placed on the warming table or in a warm oven for some hours in order that the xylol in the balsam mixture may evaporate and thus harden the gum. The hard gum has the same optical refraction as the slide and cover, and so allows light to pass through without refraction and also seals the cover to the slide.

After the gum has hardened the slide can be cleaned by dipping in toluol, draining, and permitting to dry. However, the cover should not be rubbed until the gum has thoroughly hardened.

Regressive Staining.—In this technique the sections are far overstained at first and then excess stain is extracted until just the right effect is produced. An excellent example of a well-tried stain for this purpose is the so-called Heidenhain's hematoxylin. It is highly recommended for differentiation of mitotic figures and is, therefore, indicated for studies in cell division, spermatogenesis, and oögenesis. Two solutions are used, a mordant and the stain.

To proceed with staining, the slides are placed first in xylol and brought down, as before, through aniline oil to water. The slides are then placed in a 4 per cent iron alum solution for at least an hour for thorough mordanting. Then the slides are washed thoroughly in water and placed in the hematoxylin stain for one to several hours. They will appear deep black if the staining has been effective and no details are at all evident upon microscopic examination. After rinsing in water they are placed in a 2 per cent iron alum solution for extracting excess stain, and after a minute or so examined under the microscope. It is an advantage to have the extraction take place slowly. They should be rinsed, examined, and returned if the stain is still too deep. The de-staining may also be carried out under the microscope in a glass Petri dish containing the 2 per cent alum. The chromatic network in the nucleus should appear sharply outlined. If one has a metaphase stage in mitosis, the chromosomes should appear clear-cut. When the extraction has gone far enough, it can be stopped by washing the slides in water to remove every trace of the free alum solution. Then the slides are "run up" through 50 per cent alcohol to 70 per cent alcohol. They can be counterstained in a solution of orange G or eosin in 70 per cent alcohol for thirty to fifty seconds, as in the preceding techniques. They are then transferred to 95 per cent alcohol, aniline oil, and xylol, as before, and later mounted.

If hematoxylin is not used in a slightly alkaline condition it acts partly as an acid dye, and the cytoplasm is stained reddish and the nuclei are poorly stained.

The Mallory Connective-tissue Stain.—This stain gives a colorful effect with sections that contain considerable connective tissue, muscle, and epithelium. Two solutions are needed. Solution A is a 0.5 per cent solution of acid fuchsin in distilled water. Solution B is 0.5 gram aniline blue (water solution); 2 grams orange G; 100 cc. of 1 per cent aqueous solution of phosphomolybdic acid. The stain works better with tissues that have been fixed in Zenker's fluid. After the sections have been brought down, as before, from xylol to water, they are placed in Solution A (acid fuchsin) for about four minutes. Then this is drained off and the slides are placed at once in Solution B for about eight minutes. They are then rinsed several times in 95 per cent alcohol; it is better to use more than one jar of 95 per cent. Then proceed to aniline oil, xylol, and mount as before. Connective tissue, muscle, and epithelium stain differentially, the prominent colors being red, blue, and yellow.

Staining With Silver Nitrate.—The mesothelial covering of pieces of fresh mesentery can be stained in bright sunlight with a 1 per cent solution of silver nitrate in distilled water as follows. Remove pieces of the fresh mesentery and spread carefully on a glass slide or stiff moist paper, then place in the bottom of a shallow glass dish. Wash in distilled water. Then bathe the pieces of tissue in the silver nitrate solution for about five minutes. Wash all free silver nitrate away with water and place the tissue in the water in brilliant sunshine until the membrane turns a brown color. If it is desired to stain the nuclei, this can be done by treating with Harris's hematoxylin. After passing through the various grades of alcohol, aniline oil, xylol, the tissue can be mounted on a slide in balsam. Care should be taken that the tissue does not wrinkle or fold.

STAINING CELLOIDIN SECTIONS.

We will suppose that a piece of tissue fixed in Bouin's or Zenker's solution has been embedded in celloidin and that sections of such a piece have been cut with a sliding microtome and collected in a dish in 70 per cent alcohol. We can stain these sections in the Harris hematoxylin and eosin combination as follows: Discard the 70 per cent for 50 per cent alcohol and change this to water, each bath lasting a few minutes. After the sections have been washed in water, the water can be discarded and the dish filled with hematoxylin stain. A section can be lifted out occasionally to determine the degree of staining. When the stain has taken sufficiently, it is replaced with water. From this they pass to 50 per cent alcohol; to 70 per cent alcohol; to eosin or orange G for counterstaining; to 95 per cent alcohol; to aniline oil; to cedar oil or oil of origanum. We merely decant off the liquid in the dish each time and then replace it with the next liquid. At the last step transfer a stained and cleared section to a slide with a spatula and, having added 1 or 2 drops of Canada balsam, apply a cover-glass.

PREPARATION OF STAINS.

Harris's Hematoxylin.—To prepare this stain make up two solutions independently:

    A. Add 1 gram of hematoxylin crystals to 10 cc. of 100 per cent ethyl alcohol.

    B. Add 20 grams of potassium or ammonium alum to 200 cc. of distilled water. To get the alum into solution it is necessary to heat to boiling.

When the alum solution is at the boiling-point add the hematoxylin solution. Satisfactory staining solutions have been obtained by continuing to heat the solution for about fifteen minutes after so mixing, then setting stain aside for a week to age in a loosely stoppered container before adding a few thymol crystals to act as preservative. A second method is used to make the dye ready for immediate use. In this case, 0.5 gram of yellow oxide of mercury is added as soon as the hematoxylin is added to the alum, and the solution removed from the heat. The flask is immersed in a larger vessel and cooled as quickly as possible. Before use, in either method of preparation, the solution should be filtered and a few crystals of thymol be added. This is the stock solution and may be kept for long periods in the well-stoppered bottle and improves after several weeks' aging.

The yellow oxide of mercury employed to ripen the stain does not keep well when dried, so it has to be made at the time of preparation of the solution. To prepare it, make a saturated solution of mercuric chloride by adding about 8 grams of the salt to 100 cc. of water and boil. Add to the saturated solution thus formed, about 40 grams of potassium hydroxide. A heavy precipitate is formed. Filter the solution and retain the precipitate, then wash the precipitate with distilled water until no chlorine can be detected when silver nitrate is added to the water that has passed over the mercury precipitate. This yellow paste may be kept if the filter paper upon which it is deposited is kept immersed in water. When added to the hot hematoxylin mixture it is necessary to merely approximate the weight of the paste but excess should be avoided.

The stock solution may be diluted with equal or twice its volume of water before use, depending upon the time sections are to be left to stain.

Heidenhain's Hematoxylin.—In this method of staining, the mordant and dye are kept separate, not mixed, as in the case of Harris's hematoxylin. Two solutions are prepared. Solution A is made by dissolving 4 grams of crystals of ferric alum (iron ammonium sulphate) in 100 cc. of distilled water. Solution B is prepared by dissolving 10 grams of certified hematoxylin crystals in 100 cc. of 100 per cent ethyl alcohol and then allowing to age, for several weeks at least, in a loosely stoppered container. This stock solution of hematoxylin should be diluted with water to 0.5 per cent for use in staining. Allowing the dilute solution to stand before using increases its effectiveness. For de-staining the stained sections a 2 per cent solution of the mordant may be used.

Eosin.—To prepare this cytoplasmic stain, 1 gram of eosin powder is added to 100 cc. of 70 per cent alcohol. This solution may be diluted by an equal or greater volume of the 70 per cent alcohol, if it is desirable to have the staining effected more slowly.

Orange G and Erythrosin.—Both of these dyes may be prepared in the same manner as eosin. A very satisfactory combination of their effects may be obtained by mixing about one-third of erythrosin with two-thirds of an orange G solution. So combined, sections containing connective tissue and muscle have the former stained orange and the latter a reddish color.

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GATENBY, J. B., AND COWDRY, E. V. 1928. Lee's Microtomist's Vademecum, 9th ed., London, J. and A. Churchill.

GUYER, M. F. 1927. Animal Micrology, Chicago, Ill., University of Chicago Press.

McCLUNG, C. E. 1929. Handbook of Microscopical Technique, New York, Paul B. Hoeber, Inc.

APPENDIX.

Current References.—The student should refer to the available current issues of the following journals to follow the results of the more recent contributions to microscopic anatomy and the nearly related subjects.

American Journal of Anatomy.
Anatomical Record.
Biological Abstracts.
Biological Bulletin.
Journal of Morphology.
Science.
Stain Technology.
Quarterly Review of Biology.
Zoölogical Record.

Anatomical Texts.—The following are a few of the texts which will be found useful for reference:

ADAMS, A. L. 1933. An Introduction to the Vertebrates, New York, John Wiley & Sons.

DANIEL, J. F. 1934. The Elasmobranch Fishes, 3d ed., Berkeley, Calif., Univ. California Press.

DORLAND, W. A. N. 1932. The American Illustrated Medical Dictionary, Philadelphia, W. B. Saunders Company.

ECKER, A. 1889. The Anatomy of the Frog, Oxford, England, Clarendon Press.

GOODRICH, E. S. 1930. Structure and Development of Vertebrates, New York, The Macmillan Company.

HYMAN, L. H. 1922. A Laboratory Manual of Comparative Vertebrate Anatomy, Chicago, Univ. Chicago Press.

KINGSLEY, J. S. 1926. Outline of Comparative Anatomy of Vertebrates, Philadelphia, P. Blakiston's Son & Co.

NOBLE, K. G. 1931. Biology of the Amphibia, New York, McGraw-Hill Company.

WALTER, H. E. 1928. Biology of the Vertebrates, New York, The Macmillan Company.

Histological Texts.—The following textbooks of histology will be found useful for additional reference and illustrations:

ADDISON, W. H. F. 1927. Piersol's Normal Histology, Philadelphia, J. B. Lippincott Company.

BREMER, J. L. 1927. A Textbook of Histology, Philadelphia, P. Blakiston's Son & Co.

19

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Schafer, E. S. 1934. Essentials of Histology, 13th ed., Philadelphia, Lea & Febiger.

PREPARED SLIDES.

For the introductory work in cytology and embryology, appropriate preparations are utilized.

The following list of slides are available for our students either in individual kits given to them for use throughout the term or in accessory kits for use in the laboratory:

Embryos of the frog, several sections of late stages.

Embryo of the chick, transverse sections of seventeen-segment stage in various regions.

Fetus of a bat, longitudinal sections.

Fetus of a pig, cross-sections.

Simple squamous epithelium, mesothelium of mesentery treated with silver and hematoxylin.

Retina of the eye, transverse section for pigmented epithelium.

Connective tissues:

Umbilical cord of the pig at birth, transverse section.

Umbilical cord of the fetal cat, transverse sections.

Reticular tissue in sections of lymph nodes from which lymphocytes were partially washed.

Reticular tissue in the liver or spleen impregnated with silver.

Subcutaneous tissue spread of the cat and rabbit fixed in Bouin, stained in iron-hematoxylin and eosin.

Subcutaneous tissue spread of the cat and rabbit stained for elastic fibers.

Chromatophores in section of cleared and sectioned frog lung.

Adipose tissue in the fat body of a frog.

Cornea of the eye, rabbit.

Tendon of a frog and cat, longitudinal sections.

Membranous bone of a small fetal cat.

Developing femur of a new-born rabbit.

Ground bone, cross- and longitudinal sections.

Bone-marrow of the cat.

Blood:

Smears stained with Wright's stain.

Blood of a fish, the black bass.

Blood of Amblystoma.

Blood of a turtle.

Blood of a bird, starling and grouse.

Blood of various mammals.

Muscle tissues:

Smooth muscle of the frog's intestine, dissociated and routinely stained.

Skeletal muscle and Achilles' tendon junction of the frog with chondroid tissue associated with the tendon. Longitudinal sections of Bouin fixed and iron-hematoxylin and orange G stained material.

Skeletal muscle and tendon junction of the mouse, longitudinal sections.

Cardiac muscle of the frog, dissociated and routinely stained.

Cardiac muscle in the heart of a shark, Bouin fixed, stained with iron-hematoxylin and eosin.

Cardiac muscle of the cat.

Nerve tissues:

Cerebral cortex of the cat, Golgi preparation.

Cerebellar cortex of the cat, Golgi preparation.

Cerebellum of the squirrel, Bielschowsky method and thionin stain.

Diencephalon and pituitary of a fetal cat.

Spinal cord of the cat, fixed in alcohol and stained with methylene blue (Nissl method).

Spinal cord of the cat, Golgi preparation.

Spinal cord of the frog.

Nerve fibers, routinely fixed and stained but teased, not embedded or sectioned.

Nerve trunks, cross- and longitudinal section, of the cat.

Teased nerve fibers of the frog's sciatic nerve treated with osmium tetroxide.

Blood vessels:

    Small artery of the cat and rat.

    Aorta of the dog.

    Mesenteric vein of the cat.

    Jugular vein of a bird, a hawk.

Lymph organs:

    Tonsil of the dog.

    Tonsil of human.

    Mesenteric lymph gland of the squirrel and cat.

    Cervical lymph gland of the cat and dog.

    Hemolymph gland of the rat.

    Spleen of the bullfrog.

    Spleen of Necturus, Zenker fixed and Mallory stained.

    Spleen of the water snake.

    Spleen and duodenum of a hawk.

    Spleen of a cat and dog.

    Thymus of a cat.

Integument:

    Integument of a bass (fish with ctenoid scales), celloidin embedded.

    Integument of Necturus.

    Integument of a frog.

    Wing of new-born sparrow, longitudinal section, showing skin and developing feathers together with developing bone.

    Footpad of a squirrel, embedded in celloidin.

    Human scalp, embedded in celloidin.

    Ear of a rabbit, cross-sections.

    Mammary gland of the cat, longitudinal section through the nipple.

Respiratory system:

    Gill of a dogfish, longitudinal section through the septa.

    Gill of Necturus, longitudinal section.

    Lung of Necturus, cross-section.

    Glottis and openings into the lung of the frog, longitudinal section of pharyngeal region.

    Lung of a toad and frog, cross-section.

    Trachea and lung of the water snake (Natrix), cross-sections.

    Trachea and esophagus of a bird (a grouse), cross-section.

    Lung of a hawk, cross-section.

    Larynx of the cat, cross-section.

Trachea of the rat in a cross-section of the throat.

Trachea of the cat and dog, cross-sections.

Lung of the dog, longitudinal section of a lobule.

Digestive system:

Lip of the dog.

Tongue of a fish (bass) and toad, longitudinal section.

Tongue of the frog, cross-sections.

Tongue of a rat, cross-section of the tip region.

Tongue of the cat, longitudinal section.

Tongue of the rabbit, cross-section of portion with foliate papillæ.

Tongue of the cat in the region of the vallate papillæ.

Parotid and submaxillary glands of the woodchuck and cat.

Esophagus of a frog, cross-section.

Esophagus of a turtle, cross-section.

Esophagus in a cross-section of the neck of an embryo cat.

Esophagus of a dog, cross-section of middle third.

Esophagus and stomach junction of the dog, longitudinal section.

Stomach of the dogfish, cross-section.

Stomach of a bullfrog, cross-section.

Stomach of a turtle, cross-section.

Stomach of a cat and dog, fundus region.

Stomach and duodenal junction of the dog, longitudinal section.

Small intestine of Necturus and frog, cross-sections.

Small intestine and pancreas of the water snake (Natrix).

Duodenum, jejunum, and ileum of the chipmunk, cross-sections.

Duodenum of the bat, cat, and dog, cross-sections.

Ileocecal valve of the cat and dog, longitudinal sections.

Appendix of human, cross-section.

Spiral valve of the dogfish, cross-sections.

Rectum of a frog, cross-section.

Rectum of a dog, woodchuck, and guinea-pig, cross-sections.

Cross-section of a newt (Triton) in the cloacal region, celloidin embedded.

Rectum and anal junction of the dog, longitudinal section.

Pancreas of a frog.

Pancreas of the cat, rat, and dog.

Liver of a fish (black bass).

Liver of Necturus.

Liver of the alligator.
Liver of pig, Mallory stained.
Liver of the cat and woodchuck.
Liver of the rabbit with injected bile capillaries.
Gall-bladder of an alligator.

Excretory system:

Kidney of a fish (the black bass), and ureter.
Kidney and testis of Necturus, Zenker fixed and Mallory
   stained.
Kidney of a toad, and ureter, also adrenal.
Kidney of the water snake (Natrix), and ureter.
Kidney of a hawk.
Kidney of a fetal cat showing developing metanephros and
   remnants of mesonephros.
Kidney of a cat and rat.
Ureter of an alligator.
Ureter of a dog, cross-section.
Bladder of a frog.
Bladder of a turtle.
Bladder of a dog, collapsed condition.
Bladder of a mouse, collapsed and distended conditions.
Urethra of female woodchuck, cross-section.

Female reproductive system:

Ovary of a fish (the black bass), cross-section.
Ovary of Amblystoma and Necturus.
Ovary of the water snake (Natrix).
Ovary of immature dog and cat.
Ovary of adult cat, rabbit, and chipmunk, showing corpora
   lutea, etc.
Oviduct of a skate (an elasmobranch).
Oviduct of the frog, cross-sections of several levels.
Oviduct of a turtle.
Oviduct of a cat, pig, and rabbit.
Uterus of a rabbit, resting condition.
Uterus of a cat in heat.
Vagina of a guinea-pig, cross-section.
Fetus and attached placenta of the mouse.
Placenta of a rat.

Male reproductive system:

Testis of Amblyostoma, cross-sections.
Testis of a toad.

Testis, kidney, and adrenal of an alligator (young).

Testis of the kitten, longitudinal section, showing epididymis.

Testis of the rat, cross-section. Stained with iron-hematoxylin.

Seminal vesicle of the guinea-pig, cross-sections.

Vas deferens of the dog.

Vas deferens and rudimentary Müllerian duct of the woodchuck.

Prostate of the dog, cross-section.

Penis of a chipmunk and squirrel in different regions.

Penis of a dog, cross-section of distal third.

Endocrine glands:

Pituitary of the calf, longitudinal section.

Pituitary of a kitten, longitudinal section.

Thyroid of frog.

Thyroid of a turtle.

Thyroid and parathyroid of a dog.

Adrenal of frog.

Adrenal of a dog, rabbit, and cat.

Carotid of a cat.

The slides listed above are grouped into systems; those for the tissues are supplemented by types of tissues represented in the slides listed under various systems. These slides should also be supplemented by study of fresh preparations of living tissues wherever practical and also by specially prepared demonstration slides. The majority of the slides enumerated above have been prepared in our own laboratory, but there are several supply houses from which a great variety of slides appropriate to this work may be obtained.

INDEX.

(References to illustrations given in boldface type.)