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TEXT-BOOK
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STÖHR

TEXT-BOOK
OF
HISTOLOGY

INCLUDING
THE MICROSCOPICAL TECHNIQUE

BY
DR. PHILIPP STÖHR
PROFESSOR OF ANATOMY AND DIRECTOR OF THE ANATOMICAL INSTITUTE IN ZÜRICH

SIXTH EDITION

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With 268 Illustrations

PHILADELPHIA
P. BLAKISTON, SON & CO
1012 WALNUT STREET
1896

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1896

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EDITOR'S PREFACE.

Stöhr's text-book is well known to the histologists of all nations and held in high esteem by them. To the German medical student it has become an indispensable guide. During the ten years of its existence it has reached an extraordinary sale and passed through six revised editions. It has been translated into Italian (1887), French (1890), and Russian (1891), and has thus come into the hands of the students of these nations. These facts are sufficient to guarantee the value of the work without further recommendation. Although excellent text-books of Histology already exist in English, still the peculiarity and special superiority of Stöhr's text-book justifies, in our opinion, its translation into English for the convenience of American and English students.*

It is especially intended for the use of students, but even professional histologists and physicians will find in it much valuable information, as well as suggestions for technical purposes. The chief merit of the work lies, on the one hand, in the brevity and perspicuity of the descriptive text, elucidated by illustrations which have thus far never been excelled: and, on the other hand, in the simplicity and certainty of the methods for preparing the most important microscopical specimens. The young student is thus enabled to practice histological methods privately, at a minimum cost, in connection with his courses in the university. The preparation of almost all of the specimens enumerated in the book can be made simply by means of teasing, isolation, or cutting with the razor, but those students who have a microtome at their disposal will also find, in an Appendix, brief directions for the preparatory treatment (embedding in paraffin and celloidin) of specimens for sectioning with the microtome.

With the permission of Prof. Stöhr we have made several immaterial, but for an American edition very desirable, changes in the text, and have considered it more preferable to place the technical part as a whole at the end of the book rather than in sections after the several chapters. Furthermore, we have enlarged the chapter on the Uterus, in order to give detailed consideration to the various functional conditions of the organ, and added to the book an entirely new chapter on the Placenta. Eight new illustrations (Fig. 200, 201, 202, 203, 204, 205, 206, 207) were necessary for these additions.

* In 1888 Stöhr's Text-book was utilized in Kendrick's Physiology, but in such a fragmentary form and so intermingled with selections from other authors that its chief merits were entirely lost. This use of the book cannot be considered as an English translation proper.

The editor is under great obligation to the translator, Dr. Billstein, for her successful efforts in reproducing the conciseness and clearness of the German original. Further, he desires to express his gratitude to Professor Philipp Stöhr for placing at his disposal the original electrotypes, and to Drs. Böhm and von Davidoff for the illustration of the virginal uterus (Fig. 200) from their "Lehrbuch der Histologie." He also feels deeply indebted to Professor Charles S. Minot for kind assistance, valuable criticism, and for permission to use two illustrations (Fig. 202 and 205) from his text-book of "Human Embryology"; and finally to Messrs. P. Blakiston, Son & Co., Philadelphia, for the very satisfactory reproduction of the new drawings, and for their many courtesies during the preparation of the American edition.

ALFRED SCHAPER.

HARVARD MEDICAL SCHOOL,
BOSTON, *June, 1896.*

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PART I.

GENERAL TECHNIQUE.

I. THE LABORATORY APPOINTMENTS.

1. INSTRUMENTS.

The Microscope.—I have often tested the excellent workmanship of the microscopes made in the optical workshops of *Hartnack* in Potsdam, *Leitz* in Wetzlar, *Seibert* in Wetzlar, and *Zeiss* in Jena.*

It is not advisable for the beginner to buy a microscope without previously placing it for testing in the hands of an expert. For the preservation of the microscope it is necessary to protect it from dust. When in constant use, it is best to keep it under a bell-glass in a place not exposed to sunlight. Dirt should be removed from the tube with a dry piece of soft filter-paper; from the lenses and mirror with soft leather. Balsam may be removed with a soft piece of linen dampened with a drop of alcohol. Great care must be exercised

* I advise students of the first semestre to refrain from the purchase of high-power oculars and immersion-systems. These should not be bought before entrance upon bacteriological work.

The following are recommended :—

Leitz. Catalogue No. 35, 1893. Microsc., No. V. Price 355 *M.* = \$88.75. (Without homog. Immers. and without Ocular IV, 250 *M.* = \$62.50.)

Seibert. Catalogue No. 22, 1891. Microsc., 4 a. Price 498 *M.* = \$129.00. (Without homog. Immers., Objective 3, and Ocular O, 272.5 *M.* = \$68.15.)

Zeiss. Catalogue No. 29, 1891, page 120, 9)c. Price 502 *M.* = \$125.50. (Without homog. Immers., 342 *M.* = \$85.50.) Or 10)c. Price 485 *M.* (Without homog. Immers., 324 *M.* = \$81.00.)

In the preparation of this book I have carried on the majority of the investigations with a microscope of *Leitz*.

Editor's Remark.—In the U. S. A. a duty of 35 per cent. in addition to the above prices of foreign microscopes must be paid.

Of American microscopes those from the manufactory of *Bausch & Lomb Optical Co.*, *Rochester, N. Y.*, and *New York City*, may be recommended.

For ordinary *histological* work the following is suitable :—

Stand B. B.—Ocular 1 · 3. Objectives $\frac{2}{3}$ inch and $\frac{1}{8}$ inch. (Catalogue, 1895.) Price \$62.50.

For *cytological* and *bacteriological* work a $\frac{1}{2}$ inch of *oil immersion* (price \$44.00) and an *Abbé condenser* with iris diaphragm should be added.

For convenience a double or triple *revolver* for objectives is desirable.

in the last case, lest the alcohol penetrate and loosen the balsam-setting of the lens. The lens should, therefore, be quickly washed and carefully dried. The screws of the microscope may be cleaned with benzine.

A good *razor*, flat on one side. It should always be kept sharp. The honing of it should be left to the instrument maker; but before using it each time it should be drawn without pressure over the *strop*. This razor ought to be used *only* in the preparation of thin sections.

A fine *oil-stone*.

A fine pair of straight *scissors*.

A pair of easily closing *fine forceps*, with smooth or only slightly notched points.

Four *teasing needles* with *wooden handles*, two of which may be heated, then slightly bent, heated once more, and stuck into solid paraffin, whereby they are again hardened. The other two must be kept clean and highly polished, as for the work of teasing the needles must be sharply pointed. They may be first sharpened on the stone and then polished on the *strop*. The so-called cataract-knives of the oculists are very useful.

A *section-lifter* (*spatula*), to transfer sections from fluids to the slide, is useful but not absolutely necessary. The broad blade of a dissecting knife can be substituted.

Pins, quills, cork discs, a fine *paint-brush*.

A *crayon* for writing on glass.*

Slides should be of clear glass and not too thick (1 to 1.5 mm.).

Cover-glasses measuring 15 mm. in diameter are generally large enough. Their thickness may vary from 0.1 to 0.2 mm.

Small *glass bottles* (the so-called powder-bottles), one dozen, with broad neck and a capacity of 30 and more c.c. Bottles with glass stoppers are too expensive and not desirable, as the stoppers are often poorly ground.

Some small *glass jars* with ground covers about 8 to 10 cm. high and 6 to 10 cm. in diameter.

A *cylindrical graduate*, capacity 100 to 150 c.c.

A *glass funnel*, upper diameter 8 to 10 cm.

A *pipette*. Small pipettes may be prepared by heating in a gas-flame a glass tube 1 cm. thick and 10 cm. long, pulling one end to a point, and placing on the other a small rubber bulb.

A dozen *watch-glasses* of 5 cm. diameter.

A dozen *test tubes* 10 cm. long and 12 mm. wide.

Glass rods 3 mm. thick, 15 cm. long, some drawn to a point at the end.

Old medicine glasses and bottles, which have been thoroughly cleansed, serve as receptacles for reagents.†

* If the glass is oily it must first be cleansed with *alcohol*.

† In most cases the bottles may be sufficiently cleansed with water, but sometimes it will be necessary to clean them with crude muriatic acid, or caustic lye, then with ordinary water, next with distilled water, and finally with alcohol.

Glass dishes, 10 to 12 cm. diameter, with ground covers, are not absolutely necessary, but very useful.*

In many cases these may be replaced by saucers, food dishes for birds, etc.

A few sheets of thin, white *filter-paper*, large and small gummed labels, soft pieces of linen (old handkerchiefs), a towel, a large and a small bottle-brush.

A large stone jar for refuse.

2. REAGENTS.†

General Rules.—Large quantities should not be kept on hand, for many reagents decompose in a comparatively short time. Some should be prepared just before they are to be used (*cf.* following). Each bottle must have its contents designated by a large label. It is advisable to place on the label not only the formula of the fluid referred to, but also the manner of its application. The several bottles must be closed tightly with cork or good glass stoppers. The fluid should not reach the lower surface of the cork.

1. *Distilled water* 3 to 6 liters.

2. *Normal salt solution* 0.75 per cent., aq. destill. 200 c.c., salt 1.5 gm. The cork must be provided with a glass rod reaching to the bottom of the bottle. This reagent spoils easily and must often be renewed.

3. *Alcohol*.—(a) Absolute alcohol, in quantity of about 200 c.c. The absolute alcohol of commerce is 95 per cent., and answers for most microscopical purposes. If it is desired to obtain alcohol free from water, drop into the bottle a few pieces of copper sulphate heated to white heat (15 gm. to 100 c.c. alcohol). If these become blue they must be replaced by new pieces, or be reheated. Fresh quicklime serves the same purpose, but acts more slowly.

(b) *Ninety per cent. Alcohol*.—Five hundred c.c. are prepared by diluting 475 c.c. 95 per cent. alcohol with 25 c.c. distilled water.‡

(c) *Seventy per cent. Alcohol*.—Five hundred c.c. are prepared by diluting 370 c.c. 95 per cent. alcohol with 130 c.c. distilled water.

(d) *Ranvier's 33 per cent. Alcohol*.—Thirty-five c.c. 95 per cent. alcohol and 65 c.c. distilled water.

* Most of the above-mentioned glassware (including slides) may be obtained cheaply of *W. P. Stender, Leipzig (Dampf-glasschleiferei)*, and *Dr. Bender and Dr. Hobein in München (Gabelsbergerstr.)*.

† The reagents must be obtained from a reliable chemist or well-known drug-house. Excellent stains and reagents are obtainable from *Dr. Grübler, Physiological Laboratory, Leipzig, Bayer'sche Strasse 63*.

‡ To obtain the desired per cent. of alcoholic mixtures, this ratio will serve:—

$$\begin{aligned} 100 : 95 &= x : 90 \\ \text{i. e., } 90\% , 100 : 95 &= x : 90 \\ 95 x &= 90 \cdot 100 \\ x &= \frac{9000}{95} = 94.7 \text{ or } 95 \end{aligned}$$

Therefore, to obtain 100 c.c. 90 per cent. alcohol, 95 c.c. of 95 per cent. alcohol must be mixed with 5 c.c. distilled water. For our purposes the mistakes of this ratio are too insignificant for consideration.

4. *Acetic Acid*, 50 c.c.—The official is 30 per cent.
5. *Glacial acetic acid* should be procured shortly before using (10 c.c.). That obtainable from the chemist is 96 per cent.
6. *Nitric Acid*.—A bottle should be kept on hand with 100 c.c. concentrated nitric acid of 1.18 sp. gr. (containing 32 per cent. acid hydrate).
7. Pure *hydrochloric acid*, 50 c.c.
8. *Chromic Acid*.—A 10 per cent. stock solution should be prepared (10 gm. of recently prepared crystallized chromic acid dissolved in 90 c.c. distilled water). From this can be made (a) a 0.1 per cent. chromic acid solution (10 c.c. of stock solution to 990 c.c. distilled water), and (b) a 0.5 per cent. chromic acid solution (50 c.c. of stock solution to 950 c.c. distilled water).
9. *Potassium bichromate* should be kept on hand in the following preparations: (a) 25 gm. in 1000 c.c. distilled water; (b) 35 gm. in 1000 c.c. distilled water, for the Golgi mixture (No. 11). This dissolves slowly in three to six days at room temperature. Make the solution, therefore, with warm water, or place the bottle near the stove.
10. *Müller's Fluid*.—Thirty gm. sodium sulphate and 60 gm. pulverized potassium bichromate are dissolved in 3000 c.c. distilled water. Solution can be aided by heat, as in 9.
11. *Golgi's mixture** (osmio-bichromate mixture), to be obtained by pouring together 54 c.c. of the 3.5 per cent. solution of bichromate of potash (9 b) and 6 c.c. of the 2 per cent. osmic-acid solution (18). To be prepared shortly before using. In the application of the Golgi method we need further—
12. *A solution of silver nitrate*, 0.75 per cent., which is prepared by mixing 150 c.c. of 1 per cent. solution (22) with 50 c.c. distilled water and a drop of formic acid. To be made shortly before using.†
- For "fixing" the Golgi preparations we need—
13. *Hydrochinous developer*, 5 gm. hydrochinon, 40 gm. sodium sulphate, 75 gm. calcium carbonate, 250 gm. aq. destill. From this a dilution may be made by mixing 20 c.c. of the mixture and 230 c.c. of the aq. destill. In a dark place, in a well-closed bottle, it keeps strong for weeks. The yellow color, which appears with time, does not interfere with its action.
14. *Sodium Hyposulphite* (10 gm. in 50 c.c. distilled water).—It dissolves quickly without heating.
15. *Picric Acid*.—One should keep on hand 50 gm. of the crystals and 500 c.c. of a saturated, aqueous solution, in which a certain amount of undissolved crystals must always be present on the bottom of the bottle. It dissolves easily.
16. *Picro-sulphuric Acid, Kleinenberg's Solution*.—To 200 c.c. of satu-

* *Editor's Remark*.—Cox-*Golgi mixture* is obtained by pouring together 40 c.c. of 5 per cent. solution of bichromate of potash, 40 c.c. of 5 per cent. solution of corrosive sublimate, 32 c.c. of 5 per cent. solution of chromate of potash and 88 c.c. distilled water. This mixture may be kept a long time in stock.

† Old silver solution of 0.75 per cent. not acidulated is equally useful.

rated aqueous solution of picric acid are added 4 c.c. of pure sulphuric acid. A strong precipitate forms. After an hour the mixture is filtered and diluted with 600 c.c. of distilled water.

17. *Chromo-acetic Acid*.—To 50 c.c. 0.5 per cent. chromic acid solution (8 b) are added 50 c.c. distilled water and 3 to 5 drops glacial acetic acid.

18. *Osmic acid*, 50 c.c. of 2 per cent. aqueous solution may be obtained from the chemist. (Very expensive!) It is to be kept in the dark or in a dark bottle, and when well closed will remain strong many months.

19. *Chromo-aceto-osmic Acid (Flemming's Solution)*.—Prepare a 1 per cent. chromic acid solution (5 c.c. of the 10 per cent. solution (8) to 45 c.c. distilled water). Add 12 c.c. of 2 per cent. osmic acid and 3 c.c. glacial acetic acid. This mixture is not injured by light and can be long kept in stock.*

20. *Chloride of Platinum* (expensive!).—Keep a 10 per cent. stock solution, 2 gm. dissolved in 20 c.c. distilled water.

21. *Platino-aceto-osmic Mixture (Hermann's Solution)*.—Pour into 60 c.c. of 1 per cent. solution of chloride of platinum (6 c.c. of stock solution and 54 c.c. distilled water), 8 c.c. 2 per cent. osmic solution and 4 c.c. glacial acetic acid.

22. *Silver Nitrate*.—Obtain from the chemist, a short time before using, a solution of 1 gm. argent. nitric. in 100 c.c. distilled water. To be kept in the dark or in a dark bottle.

23. *Chloride of Gold*.—Obtain from the chemist, a short time before using, a solution of 1 gm. chloride of gold in 100 c.c. distilled water. To be kept in the dark or in a dark bottle. For gold-staining we need further—

24. *Formic acid*, 50 c.c.

25. *Saturated Potash Lye* (35 per cent.) 30 c.c.—The bottle must be closed with a rubber stopper provided with a glass rod. Obtained at the chemist's.

26. *Glycerine*.—One hundred c.c. of pure glycerine is to be kept in stock and also a dilution of 5 c.c. of pure glycerine in 25 c.c. distilled water. To prevent the appearance of fungi in this mixture, add 5 to 10 drops pure 1 per cent. carbolic acid solution or a chloral hydrate crystal. The cork of the bottle should be provided with a glass rod.

27. *Bergamot Oil* (green), 20 c.c.—The oil of cloves, which is cheaper and hence frequently used, scents the whole laboratory. The bottle should be provided as above.

(a) *Xylol* to replace bergamot oil in special cases. Xylol clears up more strongly and is not to be recommended to beginners because of its sensitiveness in preparations incompletely freed from water.

28. *Damar-varnish* of Dr. Fr. Schönfeld & Co., of Düsseldorf, is purchasable in small bottles of 50 c.c. in art stores. If it is too thick it may be

* Tissues fixed with old Flemming's fluid often stain badly because the acetic acid has evaporated; 5 to 20 drops of acetic acid newly added to the solution removes this defect.

diluted with pure turpentine. It has the proper consistency if the drops, from an inserted glass rod, fall without drawing long threads. Damar is preferable to Canada balsam (diluted with chloroform), which renders the specimens too transparent. But it has the disadvantage of drying very slowly, while balsam dries quickly. The cork of the bottle must be furnished with a glass rod.

(a) *Xylol-balsam*, solution of Canada balsam, a substitute for damar-varnish.

29. *Cover-glass Cement*.—Venetian turpentine is diluted with ether sufficient to make the solution drop easily. It is then filtered warm (through a heated funnel) and the liquid inspissated on a sand bath. The proper consistency is reached when a drop transferred with a glass rod to a slide hardens at once and can be no longer indented with the finger-nail. It is better to have the cement prepared by the chemist because of the danger of fire.

30. *Böhmér's Hæmatoxylin*.—(a) One gm. of hæmatoxylin crystals is dissolved in 10 c.c. absolute alcohol. (b) Twenty gm. alum are dissolved in 200 c.c. warm distilled water and filtered after cooling. The two solutions are poured together the next day and remain standing a week in an open vessel. After the mixture is filtered it is ready for use. Cloudiness or growth of fungi in the fluid do not interfere with its action in the slightest degree. To be kept in stock. Instead of Böhmér's hæmatoxylin—

31. *Hæmalum* may be used. One gm. hæmatin-ammonium* is dissolved by the application of heat in 50 c.c. of 95 per cent. alcohol and added to a solution of 50 gm. alum in 1 liter distilled water. To this add some drops of thymol. The mixture can be used at once and keeps well.

32. *Delafield's Hæmatoxylin*.—(a) One gm. crystallized hæmatoxylin is dissolved in 6 c.c. absolute alcohol. (b) Fifteen gm. ammonium-alum are dissolved in 100 c.c. distilled water, and after cooling, filtered. The two solutions are then poured together, and the mixture left standing three days in an open vessel in the light. It is then filtered and mixed with 25 c.c. pure glycerine and 25 c.c. methyl-alcohol. After three days the mixture is filtered and may be kept a long time in stock.

33. *Weigert's hæmatoxylin* for demonstration of the medullated nerve-fibres of brain and spinal cord. One gm. hæmatoxylin is placed in 10 c.c. absolute alcohol + 90 c.c. distilled water, heated, and, after cooling, filtered. To be made ready shortly before using. The application of this stain demands the aid of a—

34. *Saturated Solution of Lithium Carbonate*.—Three to 4 gm. lith. carb. dissolved in 100 c.c. distilled water. To keep in stock. Further—

35. *A 0.25 per cent. Solution Permanganate of Potash*.—Five gm. permanganate of potash to 200 c.c. distilled water. Prepared a day before using.

36. *Acid Mixture (Pal's Mixture)*.—One gm. acid oxal. pur. and 1 gm. potassium sulphite (SO_3K_2) dissolved in 200 c.c. distilled water. This mixture is prepared a day before using and is kept in a well-closed bottle.

* To be obtained of Dr. Grübler, Leipzig.

37. *Neutral Carmine-solution*.—One gm. of the best carmine is dissolved, cold, in 50 c.c. distilled water with an addition of 5 c.c. liq. ammon. caust. The deep cherry-red fluid should be kept in an *open* vessel till it has no odor of ammonia (about three days), and then be filtered. To keep in stock. The odor of this solution immediately becomes very disagreeable but does not interfere with the staining power of the fluid.

38. *Picro-carmine*.—Add to a mixture of 50 c.c. distilled water and 5 c.c. liq. ammon. caust. 1 gm. best carmine. Stir with glass rod. After complete solution of the carmine (about five minutes), add 50 c.c. saturated picric acid solution, and leave the whole standing two days in an open vessel. Then filter. Abundant fungous growth does not diminish the staining power of this excellent medium.

39. *Alum-carmine*.—Five gm. alum are dissolved in 100 c.c. warm, distilled water and then 2 gm. carmine added. This mixture is heated from ten to twenty minutes, and, after cooling, filtered. Finally to the clear ruby-red fluid 2 to 3 drops acid. carbol. liquefact. are added.

40. *Borax-carmine*.—Dissolve 4 gm. borax in 100 c.c. warm, distilled water, and, after cooling, add 3 gm. carmine, stirring meanwhile. Then pour in this mixture 100 c.c. 70 per cent. alcohol. After twenty-four hours, filter the fluid.

Staining with borax-carmine requires after-treatment with 70 per cent. acid-alcohol, which is prepared by adding 4 to 6 drops of pure hydrochloric acid to 100 c.c. 70 per cent. (3 c) alcohol. Both to be kept in stock.

41. *Sodium Carminate*.—Two gm. of stain dissolved in 200 c.c. distilled water.

42. *Saffranin*.—Two gm. of the stain dissolved in 60 c.c. 50 per cent. alcohol (31 c.c. 96 per cent. alcohol + 29 c.c. distilled water). To be kept in stock.

43. *Eosin*.—One gm. stain dissolved in 60 c.c. 50 per cent. alcohol. To be kept in stock.

44. *Congo Red*.—One gm. color dissolved in 100 c.c. distilled water. From this stock-solution is prepared—

(a) A $\frac{1}{10}$ per cent. solution: 3 c.c. stock-solution to 100 c.c. distilled water.

45. *Nesuzin (Bismarck brown)* or—

46. *Methylviolet B* can be kept in stock in a saturated aqueous solution (1 gm. to 50 c.c. distilled water).

47. *Methylene Blue*.—One gm. dissolved in 100 c.c. distilled water.

48. *Ammonium Picrate*.—Three gm. to 100 c.c. distilled water. This keeps a long time.

49. *Westphal's Alum-carmine Dahlia*.—Dissolve 1 gm. dahlia in 25 c.c. absolute alcohol. Add 12 c.c. pure glycerine and 5 c.c. glacial acetic acid and pour into this mixture 25 c.c. alum-carmine (39). To be kept in well-closed bottles.

II. THE PREPARATION OF MICROSCOPICAL SPECIMENS.

INTRODUCTION.

Very few organs of the animal body are of a structure suitable for microscopical examination without special preparation. They must possess a certain degree of transparency which is attained either by separating the organs into their elements, *i. e.*, *isolating* the latter, or by *cutting* the organs into thin sections. On the other hand there are very few tissues which allow of cutting into very thin sections. They are either too soft, in which case they must be *hardened*, or too hard (calcified), in which case they must be *decalcified*. Fresh tissues, however, cannot immediately be *hardened* or *decalcified* without injury to their structure. Accordingly both processes must be preceded by a treatment which kills the structural elements rapidly and at the same time preserves their natural form. This procedure is called *fixation*. The preparation of fine sections, therefore, is possible only after the processes of fixing and hardening.* The sections, moreover, require still further treatment. They may first be made transparent by a *clearing agent* immediately after cutting (a process which is also successfully employed with tissues examined in a fresh condition), or they may be *stained* before being made transparent. Staining materials are *invaluable* helps in microscopical examinations. They can be applied to fresh and even to living organs. Many very important facts have been discovered by their aid. *Injected* into the blood-vessels they make evident their distribution and the course of their finest branches.

§ 1. NATURE OF THE MATERIAL.

For the study of the histological elements and the simplest tissues, amphibians (frogs, salamanders) are recommended. The best is the spotted salamander (*Salamandra maculosa*), † whose elements are very large. For the examination of the organs mammals may be chosen. Our rodents (rabbits, guinea-pigs, rats, mice, or young dogs, cats, etc.) suffice for many purposes. Still no opportunity to procure the organs of man should be neglected. Entirely fresh material can often be obtained from the surgical clinics.

In general it is advisable to place the organs in the fixing fluid while they are yet warm. To accomplish this it is recommended to first fill the bottles

* (Followed, if necessary, by decalcification).

† *Editor's Remark*.—Or the American *Amblystoma*, *Necturus*, etc.

chosen for the reception of the tissues, then to provide them with labels upon which is designated the object, the fluid used, and the date—even the hour. The instruments necessary for dissecting should be placed in order near at hand. Now the animal may be killed.*

§ 2. KILLING AND DISSECTING THE ANIMALS.

In the case of amphibians, cut with strong scissors through the vertebral column of the neck,† and destroy brain and spinal cord by means of a needle introduced into the spinal canal and cranial cavity. In the case of mammals, cut the throat of the animal by a deep incision reaching as far back as the vertebral column of the neck, or kill it by pouring chloroform on a cloth and pressing it to the nose of the animal. Embryos, small animals to the size of 4 cm., may be thrown entire into the fixing fluid. After about two hours the abdomen and thorax may be opened by means of fine scissors. In the dissection, whenever possible, an assistant should hold the extremities of the animal. Small animals can be stretched on cork- or wax-plates, fastening them by strong pins through the feet. The organs must be taken out carefully. This is best done with scissors and forceps. Crushing or pressing the parts, or taking hold of them with the fingers, is to be entirely avoided. *Only the edge* of the object should be grasped by the forceps. Attached mucus, blood, contents of the intestines, etc., should not be scraped off with a scalpel, but may be removed by slowly twirling them in the respective fixing fluids.

In the following manipulations it is inevitable to bring the scissors, forceps, needles, glass-rods, etc., in contact with the different fluids, *e. g.*, acids. The instruments should therefore be cleaned *immediately* after using by rinsing them in water and drying them. Above all, avoid dipping a glass-rod which may be contaminated, for instance, with acid or dye, into another fluid. It is obvious that the reagents would thereby be spoiled. The success of the preparation is often entirely frustrated by such inaccuracy. The jars, watch-glasses, etc., are easy to clean if attended to directly after using. If, however, there is not time for this, the watch-glasses should be at least thrown into a dish of water.

All vessels used for isolating, fixing, hardening, or staining must be kept closed. (A watch-glass is covered with a second one, if the work with it extends beyond ten minutes.) They should not be placed in the sun.

§ 3. ISOLATING.

The process of isolation is accomplished by teasing either the fresh tissues or those previously treated with macerating fluids. In the latter case the isolation has been entirely or in part accomplished by action of the fluid. It is a difficult task to make a well-teased preparation. Great patience and exact

* To take parts from the *living* animal is an entirely needless cruelty!

† In doing this, hold the frog's hind legs with a cloth in the left hand.

obedience to the following directions will be indispensable. The needles must be sharp and perfectly clean. They should previously be pointed on a moistened whet-stone. The *small* piece of tissue, at the most 5 mm. on a side, is now placed in a small drop on a slide and teased on a dark background if it is colorless, on a white surface if it is dark (or colored). If the tissue is fibrous, *e. g.*, a muscle-fibre bundle, both needles are first applied to one end, and the bundle broken in two, lengthwise, by gradually separating the points.*

In the same manner one of these half-bundles is again divided, and so on until fine single fibres have been isolated. By examination of the uncovered preparation with low power we may ascertain whether the necessary degree of isolation has been attained.†

As isolating fluids the following are to be recommended:—

(a) *For Epithelium*.—Ranvier's 33 per cent. alcohol (p. 19), an excellent medium for isolation. Lay small pieces from 5 to 10 mm. on a side, *e. g.*, intestinal mucous membranes, into about 10 c.c. of this fluid. After four hours (in the case of stratified pavement epithelium, after ten to twenty-four hours or later) the pieces are taken out *slowly and carefully* with the forceps, and struck *lightly* a few times upon the slide in a drop of the same fluid. As a result of this striking many cells drop off either isolated or in shreds, which latter may be broken up by stirring the fluid slightly with the needle. Now apply a cover-glass (p. 38) and examine. If staining of the specimen is desired, the *entire* piece must be carefully transferred from the alcohol into about 6 c.c. picro-carmin (p. 23). After two to four hours the piece is placed very carefully in 5 c.c. distilled water, and after five minutes struck upon the slide, this time in a drop of dilute glycerine (p. 21). Apply a cover-glass. The preparation can be preserved.

(b) *For Muscle Fibres, Glands*.—Thirty-five per cent. potash lye is suitable (p. 21). Pieces from 10 to 20 mm. a side may be laid into 10 to 20 c.c. of this fluid. After about an hour the pieces fall into their elements. These may then be lifted out with a needle or a pipette, and examined under a cover-glass in a drop of the same lye. Diluted potash lye works quite differently, as in this medium the elements are destroyed in a short time. If the isolation is not successful, but instead a pappy softening occurs, the lye is too old, and a fresh solution should be made. The preparations, even when successful, cannot be preserved.

A mixture of potassium chlorate and nitric acid may be used. Prepare this by throwing into 20 c.c. of pure nitric acid (p. 20) about 5 gm. of potassium chlorate, which will be sufficient to allow an undissolved portion to remain at the bottom. After one to six hours, often longer, the specimen is

* At times it is difficult to divide the bundle along its entire length. In this case, it is often sufficient to break up only $\frac{3}{4}$ of its length, so that the isolated fibres still hang all together at the other end.

† Specimens lying in a small amount of fluid and not covered with a cover-glass appear often indistinct, show black outlines, etc. By the addition of more fluid and the use of a cover-glass these conditions are removed.

sufficiently macerated, and is then immersed in 20 c.c. distilled water. Here it should remain an hour, but may stay a week without decomposing. Then it is transferred to the slide, on which, in a drop of dilute glycerine, it can be easily teased. When the nitric acid is well washed out, the preparation may be stained under the cover-glass (p. 41), and preserved. Placing the unteased pieces in picro-carmin (see the isolation of epithelium) will not be successful, because this staining fluid renders the objects brittle.

(c) For *gland-tubules*, place the small pieces, 1 cm. a side, in 10 c.c. of pure hydrochloric acid. After ten to twenty hours they are transferred into 30 c.c. distilled water, which must be changed several times within twenty-four hours. It is then an easy matter to bring about the isolation by carefully spreading the small pieces with a needle in a drop of dilute glycerine. The specimens may be preserved.

§ 4. FIXATION.

General Rules.—1. For fixing, the quantity of the fluid used should exceed 50 to 100 times the volume of the specimen to be preserved. 2. The fluid must always be *clear*. As soon as it becomes clouded it must be replaced by fresh fluid. The clouding often begins within an hour after the introduction of the specimen. 3. The specimens to be preserved should be *as small as possible*; in general, not exceeding 1 to 2 c.c. Should the fixation of the entire object be necessary (*e. g.*, for after-orientation), make many deep incisions into it, one to two hours after it has been introduced. It should not lie in the fixing fluid on the bottom of the vessel, but be suspended in the glass, or placed upon a layer of cotton or glass-wool.

1. *Absolute alcohol* is very suitable for glands, skin, blood-vessels, etc. It acts at the same time as a means of hardening. Specimens fixed in absolute alcohol can be sectioned after twenty-four hours.*

On this account alcohol is especially suitable for *quick* preparation of microscopical specimens. The following points should be especially noted: 1. The absolute alcohol must be changed after three to four hours, even when it is not clouded. 2. Avoid allowing the objects to lie flat upon the bottom of the vessel, or to attach themselves to it.† They should either be suspended by a thread in the alcohol or laid on a small pad of cotton on the bottom of the glass.

Weaker alcohol (*e. g.*, 90 per cent.) acts quite differently, shrivelling the tissues, and, therefore, cannot be employed in the place of absolute alcohol.

2. *Chromic acid* is mainly used in two aqueous solutions:—

(a) As 0.1 to 0.5 per cent. solution (p. 20), which is especially suitable for organs which contain a great amount of loose connective tissue. This strong

* One should not too long delay using objects preserved in absolute alcohol, for the elements deteriorate gradually. They may be sectioned after three to eight days. Sections of specimens which have lain only twenty-four hours in absolute alcohol are likely to stain poorly.

† The portion which has thus been in contact with the vessel appears strongly compressed in the sections.

solution gives to the connective tissue a superior consistency, but has the disadvantage of making the staining difficult. It is further useful for fixing the karyokinetic figures. The specimens remain here one to eight days, after which they are placed three to four hours in running water, or, if that is not possible, for the same period in water changed three to four times. Then they are transferred to distilled water for several minutes and at last hardened in gradually strengthened alcohol protected from daylight (§ 5).

(b) As 0.05 per cent. solution, which is prepared by diluting the 0.1 per cent. solution with an equal amount of distilled water. Treat as under solution (a), except that the objects remain only twenty-four hours in solution (b).

Chromic acid solution penetrates slowly. Therefore, only small pieces of 5 to 10 mm. a side should be preserved.

3. *Nitric acid* as 3 per cent. solution (3 c.c. concentrated nitric acid [p. 20] to 97 c.c. distilled water) is, like the strong chromic acid solution, an excellent medium for organs rich in connective tissue. The objects remain five to eight hours in this solution, and are then carried, not into water, but directly into alcohol of gradually increased strength, for hardening.

4. *Kleinberg's Fluid* (p. 20).—Delicate objects (embryos) may be allowed to remain five hours in this fluid, and more solid parts twelve to twenty hours. Then for hardening they are transferred to gradually strengthened alcohol (§ 5) without previous washing.

5. *Müller's Fluid* (p. 20).—The objects are placed, for one to six weeks,* in a large quantity (— 400 c.c.) of this solution, then for four to eight hours washed in running water, rinsed in distilled water, and finally brought into gradually strengthened alcohol protected from daylight (p. 29, foot-note). If one fails to follow with painstaking conscientiousness the above-specified rules for fixing, he will secure imperfect results, for which even experienced microscopists have held the blameless Müller's fluid responsible.

6. *Osmic Acid Solution* (p. 21).—In the use of this, care should be taken not to inhale its irritating vapor. Fix the specimens, either by placing the moist pieces in the vapor or by immersing minute pieces (to 5 mm. a side) in a small quantity (1 to 6 c.c.) of the acid (most used in 1 per cent. solution). To fix by means of the vapor, fill a test-tube of 5 cm. depth with 1 c.c. of the 2 per cent. solution and the same amount of distilled water, and fasten the specimen with quills to the underside of the cork of the test-tube. After ten to sixty minutes, according to the size of the specimen, it is taken from the cork and placed in the fluid in the test-tube. In both cases the objects stay twenty-four hours in the acid, and, during this time, the tube must be well-closed and kept in the dark. Then the objects are taken out, washed in running water for one-half to two hours, rinsed quickly in distilled water, and hardened in alcohol of gradually increased strength (§ 5).

7. *Chromo-aceto-osmic acid* (*Flemming's solution*) (p. 21) is an excellent

* One can keep the pieces still longer—up to six months in Müller's fluid. They can then be cut and stained without the alcohol hardening.

medium for fixing karyokinetic figures. Place absolutely fresh pieces, still *warm* and from 3 to 5 mm. on a side, in 4 c.c. of this fluid where they remain one to two days or even longer. Then the pieces should be washed in running water for one hour (or better longer), rinsed in distilled water, and hardened in gradually strengthened alcohol (§ 5).

8. *Platino-aceto-osmic mixture* (*Hermann's solution*) is very useful for displaying sharply defined cell boundaries. It is used as Flemming's fluid.

The fluids used for fixing cannot be used again, but must be thrown away.

§ 5. HARDENING.

Except when absolute alcohol is used the above methods of fixing necessitate a supplementary process of hardening. The best means of hardening is by *gradually strengthened* alcohol. It is necessary to use abundant fluid and to change the alcohol as it becomes cloudy or colored.*

The following is the exact treatment: After the specimens have been fixed in one of the above-enumerated fluids and washed in water,† they should be transferred for twelve to twenty hours to 70 per cent. alcohol and then placed in 90 per cent. alcohol, where, after twenty-four to forty-eight hours more, the hardening is completed. In this alcohol the specimens may remain for several months, until one is ready to use them. The 90 per cent. alcohol, after it has been used for hardening, can be collected for burning or for hardening liver for imbedding.

§ 6. DECALCIFYING.

The specimens to be decalcified cannot be laid fresh into the decalcifying fluid, but must first be fixed and hardened. For this purpose, lay, in 300 c.c. of Müller's fluid, small bones up to the size of a metacarp, entire teeth, and pieces 3 to 6 cm. in length sawed from the larger bones. After lying for two to four weeks in Müller's fluid and having been washed, these pieces are placed in 150 c.c. of gradually strengthened alcohol. After remaining three days, or longer if necessary, in 90 per cent. alcohol, they are transferred into the decalcifying fluid, *i. e.*, dilute nitric acid (pure nitric acid 9 to 27 c.c. to 300 c.c. distilled water). Large quantities (at least 300 c.c.) of this fluid should be used, being changed at first *daily*, and later every four days until the decalcification is complete. The degree of decalcification may be ascertained by cutting with an old scalpel.‡ Decalcified bone is elastic,

* If not washed for a long time (and that must be avoided because of the danger of decomposition) the pieces, preserved in chromic acid and in Müller's fluid, under the action of daylight form a precipitate. The alcohol, therefore, must be kept in the dark so that no precipitate shall be formed. In that case the alcohol, though remaining clear, becomes yellow. The 90 per cent. alcohol must also be changed daily as long as it becomes intensely yellow.

† An exception is made only to those objects which have been fixed in 3 per cent. nitric acid and in picrosulphuric acid. These should be transferred directly to 70 per cent. alcohol, which must be changed several times during the first day.

‡ The scalpel should be at once carefully cleaned.

soft, and can be easily cut. Fœtal bones, heads of embryos may be decalcified in weaker nitric acid (1 c.c. of pure acid [p. 20] to 90 c.c. distilled water) or in 500 c.c. saturated aqueous picric acid solution (p. 20). The decalcifying process requires several weeks with thick bones; but only three to twelve days with small and fœtal bones. As soon as the decalcifying is completed, the bones are washed six to twelve hours in running water and afterward hardened in gradually strengthened alcohol (§ 5).

Beginners, not seldom, transfer the bone to alcohol before the decalcification is complete, so that it cannot be used for sectioning. In such cases the entire decalcification process must be repeated. On the other hand, specimens left too long in the decalcifying fluid, will be entirely destroyed.

§ 7. SECTIONING.

The razor (p. 18) must be sharp, for success in sectioning depends largely upon the good condition of the knife. In cutting, the blade must be wet with alcohol. (Water is not suitable for this purpose for it does not adhere evenly to the surface of the blade.) The razor should, before every third or fourth section, be dipped into a flat dish, filled with 30 c.c. 90 per cent. alcohol, which serves at the same time as a receptacle for the sections which have been prepared. The razor is to be held lightly, in a horizontal position, with the thumb toward the sharp edge, the fingers toward the back of the blade and the back of the hand upward. First a smooth surface of the object to be sectioned must be made by a single, rapid, and even cut of the razor. From this surface a large number of sections, one after another, may be prepared, as thick as desired. The sections should always be cut with a light, not too quick, movement, as smooth and as evenly thin as possible. *

It is always desirable to make ready a large number (10 to 20) of the sections, which may be transferred to a glass dish by floating them, with the aid of a needle, off from the blade immersed in the fluid. †

Then place the dish on a black surface and search out the best sections. The thinnest sections are not always the most useful. For many preparations, *e. g.*, for sections through the coats of the stomach, thicker sections are to be recommended. For a general view, large thick sections should be prepared; for the study of finer structures, the thinnest possible sections. If the object to be cut is too small to be handled in the fingers, then it should be imbedded. A simple method of imbedding is by pressing the specimen into hardened liver. For this may be used either ox liver or, better, human fat- or amyloid-liver (to be obtained from the pathological laboratory). ‡ This should be cut into pieces

* In doing this the edge of the razor should not be pressed against the object but gently drawn through it from right to left.

† Very fine sections, if they are not to be stained or if they are already stained, can best be drawn or rinsed from the inclined edge of the razor directly upon the slide.

‡ Dog's liver (to be obtained from the physiological laboratory) is also recommended.

3 cm. high, 2 cm. broad, and 2 cm. long, and thrown immediately into 90 per cent. alcohol, which must be changed the next day. In three to five days the liver has gained the necessary hardness. Now in one of these pieces an incision should be made from the top to the centre and into this the specimen should be pressed. If the specimen is too thick, the incision may be enlarged with a small scalpel. The object needs no further fixing, other than perhaps binding together with thread.

I am accustomed to imbed most specimens in hardened liver. One can thus make very thin sections, at least with a certain amount of practice which can be acquired in a few weeks.

§ 8. STAINING.

Before using a stain it should always be filtered. A small funnel may be made by simply folding twice a piece of filter-paper 5 cm. sq. and sticking it in a cork frame. To make this frame, cut a piece 2 cm. sq. from the centre of a cork-plate 5 cm. sq. The cork frame should be placed on four long pins. Such a funnel and frame can be used many times, but only for the same fluid. The sections should not swim on the surface of the staining fluid, but be submerged with needles.

1. *Nuclear Staining with Böhmér's Hæmatoxylin* (see p. 22).—Filter 3 to 4 c.c. of the staining fluid into a watch-glass and place the sections therein. The time required for staining varies greatly. Sections fixed and hardened in alcohol stain in one to three minutes. If fixed with Müller's fluid they must remain somewhat longer (to five minutes).*

From the stain place the sections in a watch-glass in distilled water, rinse (*i. e.*, move them about somewhat with a needle to free them from the surplus stain), and after one to two minutes transfer them to a large dish filled with 30 c.c. distilled water. Here they should remain at least five minutes until their blue-red color has gradually changed to a bright dark blue. This color will be the purer the longer the sections are allowed to remain in water (even to twenty-four hours).†

Beginners are recommended to leave the sections different lengths of time, one, three, five minutes, in the stain in order to note which time gives the best staining. The chief essential in hæmatoxylin staining is proper rinsing. If the water becomes blue, then it must be renewed. The used stain may be

* Sections fixed in strong solution of chromic acid or those not entirely free from acid, stain very slowly, at times not at all. One can remedy these defects by either keeping the specimens two or three months in 90 per cent. alcohol, changed two or three times, or placing them five or ten minutes in a watch-glass with 5 c.c. distilled water, having added 3 to 7 drops of 35 per cent. caustic potash. Then transfer the sections to a watch-glass with fresh distilled water for one or two minutes, and from thence to hæmatoxylin. After five to ten minutes these sections will stain also.

† At first the sections have a *diffuse* blue tint. The differentiation takes place usually after five minutes, sometimes not for hours.

poured back into the hæmatoxylin bottle through filter-paper. The watch-glass should be cleaned at once.

Hæmalum (p. 22) appears to render still better service, since it never over-stains. It is used in a similar manner, requiring, however, a somewhat longer time for staining, often as much as ten minutes.

2. *Nuclear Staining with Alum Carmine* (p. 23).—Filter 3 to 4 c.c. of stain into a watch-glass and place the sections therein for at least five minutes. The advantage of this stain lies in the fact that the sections can remain somewhat longer in the solution without becoming overstained, as easily happens in hæmatoxylin. A disadvantage is that the alum carmine is a pure nuclear stain, while hæmatoxylin gives the protoplasm also a grey or grey-violet tint, thereby rendering it easily recognizable.

3. *Diffuse Staining*.—For staining protoplasm and intercellular substance :

(a) *Slow Stain*.—A small drop of neutral carmine solution (p. 23) is brought by a glass rod to a dish filled with 20 c.c. distilled water, on the bottom of which lies a small piece of filter-paper.*

The sections stay over night in the fluid. The weaker the stain the longer the sections need to remain in it and the more effective the staining. The beginner is always inclined to consider the pale pink solution too weak to produce a good stain, until on the next day the dark pink or red sections teach him better.

This stain by itself can be used in only a few cases, but is to be recommended for double staining. In the latter case stain first with the carmine solution and then with hæmatoxylin.

(b) *Rapid Staining*.—Add 10 drops of the eosin solution (p. 23) to 3 to 4 c.c. distilled water. The sections remain one to five minutes in it, then are quickly rinsed in a watch-glass with distilled water (see Hæmatoxylin Staining) and placed for ten minutes in 30 c.c. distilled water. The stain may be used alone or combined with hæmatoxylin, in which case the whole process of hæmatoxylin staining is to be carried out first and then that of eosin staining.

4. *Staining of the Chromatin Substance* (for karyokinetic figures).—The specimens are placed for five to ten minutes in a dish with 10 c.c. distilled water and one drop of pure hydrochloric acid; next washed off in distilled water one minute, then laid for five minutes in a watch-glass of safranin solution (see p. 23). The sections or membranes are taken out with a needle and placed in 5 c.c. absolute alcohol, to be differentiated. When the sections give off no more color (usually in one and a half to two minutes) they are transferred to 5 c.c. fresh absolute alcohol, and, after a minute longer, are cleared and mounted in balsam (see § 10, 3, p. 39). A stay too prolonged in the absolute alcohol may lead to total decolorization. Unsuccessful staining is due usually to an insufficient amount of acetic acid in the Flemming's fluid (p. 21, foot-note).

5. *Staining in Bulk*.—Nuclear staining of the whole specimen before sectioning.

The fixed and hardened specimens are placed in 30 c.c. borax-carmine for

* If the filter-paper is omitted the sections stain only on one side.

twenty-four hours if they are small (5 mm. square) or for two to three days if they are larger. (The used stain may be returned to its bottle.) From this they are transferred directly to 25 c.c. 70 per cent. acidulated alcohol (p. 23) which, after a few minutes, grows red* and must then be renewed.

After about fifteen minutes the alcohol is again changed. This is repeated until the fluid is no longer colored. † The piece is then transferred to 90 per cent. alcohol, and if it is not, after twenty-four hours, hard enough to cut, it is placed for another twenty-four hours in absolute alcohol.

6. *Picrocarmine. Double Staining.*—Nuclei and connective tissue red, protoplasm yellow.

About 5 c.c. of the fluid are filtered into a watch-glass. The length of time necessary for the action of picrocarmine is very different for individual objects and can be given approximately only in the special directions. At the end of the process the stain is filtered back into the bottle and the specimens transferred to 10 c.c. distilled water for ten to thirty minutes. (This rinsing is omitted in staining under the cover glass; p. 41.) If the specimen (*e. g.*, a section) is to be dehydrated in absolute alcohol (see p. 39), it should not remain longer than one to two minutes, as the alcohol extracts the yellow stain. ‡

Picrocarmine is used chiefly in examining fresh specimens. If the solution is good, a very pretty staining is obtained and the outlines may be yet more sharply brought out by the addition of acid glycerine (see p. 41).

7. *Nuclear Staining with Aniline Dyes.*—The best aniline-stains for this purpose are *resorzin* and *methylviolet B*. (See p. 23.)

Filter 5 c.c. of the fluid into a watch-glass. The sections placed in this stain become quite dark in two to five minutes, and are then washed off quickly in 5 c.c. distilled water and transferred to a watch-glass of absolute alcohol, where they give off much color. After a few minutes (three to five) the sections become clearer, and even with the unaided eye certain parts, *e. g.*, the glands in the skin, may be recognized. Now the sections are placed in a second watch-glass of 5 c.c. absolute alcohol, and after two minutes cleared and mounted in damar-varnish (p. 39). The result is a very beautiful permanent nuclear staining. A disadvantage lies in the necessity for using so much alcohol.

Saffranin can be similarly employed. The sections stained for five minutes are rinsed quickly (one-half minute) in a watch-glass with absolute alcohol, and then transferred to a second glass of absolute alcohol, which must be renewed as soon as it becomes an intense red. After five to fifteen minutes

* Specimens fixed in Müller's fluid give off very little stain.

† These changes may require one to three days. The alcohol should be renewed every two hours the first day and every four hours thereafter. If one wishes to be economical he can slowly push the specimen with a needle out of the red fluid area in which it lies into an unstained portion of the fluid.

‡ One can prevent the decolorization by throwing into the watch-glass of absolute alcohol a small picric-acid crystal.

(the time varies with the thickness of the sections) the sections become lighter, and are then cleared and mounted in damar-varnish (p. 39).

8. *Methylene blue* for staining axis-cylinders.

The method is to be used only for absolutely fresh specimens. Prepare a dilute ($\frac{1}{15}$ per cent.) solution by adding 15 c.c. distilled water to 1 c.c. of the 1 per cent. solution (p. 23). From this a few drops are applied to the fresh preparation lying upon the slide and lightly covered with a watch-glass.*

After one to one and one-half hours the reaction begins. To prevent drying during this period add from time to time a drop of the diluted staining solution, or of normal salt solution. Then put on the cover-glass. By this process the axis-cylinder stains a beautiful blue color. Still other elements, as nuclei, connective-tissue fibres, etc., and, if the specimen remains longer in the solution, even the medullary sheaths of the nerves are stained. For preserving the preparation, the staining solution is replaced after the method given on p. 41, by a few drops of a solution of picrate of ammonia (p. 23), the blue color changing thereby to violet. Then a drop of glycerine is allowed to pass under the cover-glass. After eighteen to twenty hours still more solution of picrate of ammonia is added to the glycerine, and the cover-glass secured by cement (p. 22). The preparations must not be left long in the sunlight, or they will fade, and, in any case, soon lose their original beauty.

9. *Mucous staining with Delafield's hæmatoxylin.*

Filter into a watch-glass filled with 25 c.c. distilled water 3 drops of this hæmatoxylin (p. 22). In this dilute solution the sections (best those specimens fixed in Flemming's fluid)† are placed for two to three hours.

Usually after this period the mucus, *e. g.*, in goblet-cells, is already an intense blue, which can be ascertained by examining even with low power the sections as they lie in the solution. It is often necessary for the sections to remain a long time in the solution. Then the sections are rinsed one minute, and, after the rule given (§ 10, 3, p. 39), mounted in damar-varnish. The nuclei stain blue also. A very pretty effect is produced in combination with safranin and picric acid. This

10. *Triple staining* is obtained in the following manner: The sections stained in Delafield's hæmatoxylin are transferred for five minutes to safranin (p. 23), then to 5 c.c. absolute alcohol, which is changed twice in fifteen minutes. Then the sections are placed for a minute in 5 c.c. absolute alcohol, to which is added 5 drops of saturated alcoholic picric acid solution (1 gm. picric acid to 15 c.c. absolute alcohol). They are then rinsed in pure absolute alcohol one-half minute and, after rule § 10, 3, p. 39, mounted in damar-varnish. Result: mucus blue, nuclei red, protoplasm, fibres yellow.

*The cover-glass should not be closed hermetically, for the access of air is necessary for the success of the staining. By gently moving the preparation to and fro the action may be started.

† Specimens fixed in Müller's fluid are also suitable for mucus staining.

11. *Silver Staining*.—For exhibition of cell outlines, staining of the inter-cellular substance.*

The use of metal instruments is to be avoided. Use glass rods and instead of needles take quills.

The specimen is placed in 10 to 20 c.c. of 1 per cent. or weaker (see Special Technique) solution of silver nitrate (p. 21) for one-half to ten minutes, according to its thickness. It is then taken with glass rods out of the fluid, which has meanwhile become milky, rinsed, placed in a large white dish (a porcelain plate) with 100 c.c. distilled water, and exposed to direct sunlight. After a few minutes a slight browning appears, the sign of successful reaction. As soon as the specimen becomes a dark red-brown, usually after five to ten minutes, it is taken out, transferred for five to ten minutes to a watch-glass with distilled water to which a few grains of common salt have been added. It is then placed in 30 c.c. 70 per cent. alcohol in the dark. After three to ten hours the 70 per cent. is replaced by 90 per cent. alcohol. The specimen must be kept away from the light while in the silver solution. The reduction on the contrary should only be brought about in direct sunlight. †

If the sun does not shine, place the specimen, which has been taken out of the silver solution and quickly rinsed in distilled water, in the dark in 30 c.c. 70 per cent. (later 90 per cent.) alcohol, and it may be exposed to the next sunlight.

Golgi's method‡ for demonstration of the elements of the nervous system.

The method unites fixing and staining. The objects must be as fresh as possible. Their diameter should in general not exceed 4 mm. It is, however,

* Crossmarkings, which under treatment with silver nitrate appear in the different tissue elements and organs, especially in nerve-fibres, blood-vessels, in cartilage, etc., are artificial products of the stiffening and acidifying action of silver nitrate upon colloid structures.

† The reduction takes place also in ordinary daylight, but only slowly, and shows less distinct outlines.

‡ *Editor's Remark*.—In American laboratories a modification of the Golgi method, by Cox, is often used with excellent results. This method may be specially recommended to beginners, because its management is very simple and almost always successful. Unfortunately this method is not applicable for younger embryonic tissues. In using it the following directions must be observed: Put pieces, 2 centimeter cubes or smaller, of the central nervous system of adult or new-born animals into the *Cox-Golgi mixture*, whose composition is given on p. 20, *remark*, for six to ten weeks. Use 10 to 20 times the volume of the tissue to be hardened. Change the fluid after twenty-four hours, three days, eight days, fifteen days, twenty-one days, and thirty days. Keep the pieces in the fluid till ready to cut. Specimens will keep in good condition about ten months. The preparations for cutting are the following: Put the pieces from the Cox-Golgi mixture directly in alcohol of 95 per cent. for one hour, in alcohol-ether (equal parts) for half an hour, in thin celloidin solution (in alcohol-ether) for one hour. Then mount the pieces on a block in celloidin (see Microtome Technique) and harden in alcohol of 80 per cent. for one to two hours. Cut, at once, rather thick sections (50 to 100 μ). Clear the sections in a mixture of xylol 3 parts and phenol 1 part, in which they may be kept for weeks without injury. Mount in balsam and cover the sections with a cover-glass. After a time the specimens thus preserved are not seldom marred by the appearance of corrosive crystals, but the impregnation of the elements of the nervous tissue keeps in good condition.

not easy to cut pieces of this size of brain and other soft objects without crushing the delicate tissue. For this reason, larger pieces (3 cm. a side) should be laid in a dish of Golgi's mixture freshly prepared (p. 20), covered and set in the dark, in winter in a warm chamber at 25° C. After one to two hours the pieces may easily be cut into slices 4 mm. in diameter. The amount of Golgi fluid is to be regulated after the number of slices, each slice requiring about 10 c.c. After two to six, seldom as many as fifteen days* the slices should be taken out, rinsed rapidly a few seconds in distilled water, dried gently on filter paper and laid † in acidulated silver solution (10 c.c. for each slice). A brown precipitate covers immediately the pieces. It is sufficient to leave them two days in the silver solution, which does not need to stand in the dark and *must not* be placed in the warm chamber. The pieces can, however, remain therein for six days without injury. Then they go into 20 c.c. absolute alcohol for fifteen to twenty minutes (not longer). They are then imbedded in elder pith (or in celloidin, see Microtome Technique) and cut in thick sections (p. 30).

In order to ascertain which may be utilized each section is examined at once, without cover-glass and with low power, and if suitable transferred to a watch-glass of absolute alcohol one to two minutes, next to creosote two minutes, and then to bergamot oil two minutes. From this, the section goes a few seconds in xylol, and then upon the slide. The xylol may be removed by gently pressing filter paper on the section. A drop of balsam which is thinned with xylol is then added to the preparation. A cover-glass must not be laid on it because thereby the moisture in the preparation is prevented from evaporating which spoils the Golgi preparations. Occasionally, when the xylol is not sufficiently removed, the Canada balsam gradually withdraws from the preparations. These appear to be spoiled, but by adding another drop of balsam they are completely restored. Examine first with low power. When the balsam is dry the high power can be used.

The results with this method, if successful, are excellent. Single elements of the nervous system (never all) stand out sharply black on a light background. But the method is also accompanied with undesirable effects. At times blood- and lymph-vessels, fibres of connective tissue, secretion, muscular fibres, and epithelial cells are blackened and almost always the best sections are disfigured by a black precipitate. Fortunately this appears on the edges of the preparations. To avoid this the fresh specimens may be furnished with a coating of clotted blood. Very often the reaction fails entirely, especially when the specimens have remained too long in the Golgi mixture. Then the so-called "double method" may be applied, that is to say, the specimens (if the first sections show nothing) are placed a second time in the Golgi mixture for twenty-four to thirty-six hours and just as long in the silver solution.

* For this see special directions.

† The used Golgi mixture is to be thrown away.

After a second failure we may sometimes succeed by a third repetition of the process. Practice and patience are important factors in the use of this method.

One can also "fix" the preparations blackened in this way. For this purpose the sections are taken from the alcohol and placed in a mixture of 10 c.c. absolute alcohol* and 20 c.c. of diluted hydrochinin solution for five minutes, where they become dark gray to black. When the reduction is accomplished the sections are transferred directly to a dish of 70 per cent. alcohol for ten to fifteen minutes. There they become lighter, and then go for five minutes into the soda solution (p. 20.), and at last into a large dish of distilled water, where they must remain at least twenty-four hours. Thus treated, Golgi preparations will last like any other preparations and can be preserved under a cover-glass. They may be successfully stained with alum carmine and hæmatoxylin.

13. *Gold Staining*.—For demonstration of nerve-terminations.

Steel instruments should not be used. All manipulation in the gold solution are to be performed with glass needles or rods.

Heat in a test-tube 8 c.c. of the 1 per cent. gold chloride solution 2 c.c. formic acid to boiling point three times. Into the cooled mixture very small pieces (largest 5 mm. a side) are laid for an hour (to be kept in the dark), then quickly rinsed in a watch-glass in distilled water and set in the light (not sunlight) in a mixture of 10 c.c. formic acid and 40 c.c. distilled water. (Thereby the pieces become dark violet on the outside.) The reduction follows very slowly, often after twenty-four to forty-eight hours. The pieces are then transferred to 30 c.c. 70 per cent. alcohol, and, on the following day, placed in an equal quantity of 90 per cent. alcohol, where, to hinder a further reduction, they must remain in the dark at least eight days.

§ 9. INJECTING.

Filling the blood- and lymph-vessels with colored matters is a special art which can be acquired only by a great deal of practice. Many of the little devices used can hardly be learned from written directions, however explicit. Here practical knowledge is indispensable. Therefore, I think it better to omit entirely extensive directions for injecting in this book, which is intended only for beginners.

He who will attempt to inject must have an exactly-working hand-syringe, provided with easy-moving piston and canulæ of different thicknesses. As injecting material I recommend Berlin blue of Grübler (p. 19), 3 gm. dissolved in 600 c.c. distilled water. It is best to begin with the injection of a single organ, *e. g.*, the liver, which has the advantage of furnishing useful results, even though it is imperfectly filled. Fix the injected objects two to four weeks in Müller's fluid (p. 28), and harden them in gradually strengthened alcohol (p. 29). The sections should not be cut too thin.

* If one takes too much alcohol a precipitate appears, which, by addition of hydrochinin solution, can be quickly removed.

§ 10. MOUNTING AND PRESERVING OF THE PREPARATIONS.

The sections treated after the above-given directions are now ready to be transferred for microscopical examination to a slide and to be provided with a cover-glass. The fluids in which the sections are placed for this purpose are either: 1, *water*, or (if one desires to clear or preserve the sections), 2, *glycerine*, or, 3, *damar-varnish*.

To transfer the specimen to the slide, first place a small drop of the fluid to be used on the middle of the slide; then take the specimen with the section lifter and slip it off with needles upon the slide. Very fine sections are lifted better with the end of a glass rod, by rolling which they are brought on to the slide. The section will then lie out smoothly and may be covered with a cover-glass.*

The cover-glasses must be grasped edgewise, not touching the surface. In covering the specimen the cover-glass should be set down with the left edge upon the slide, and then slowly lowered upon the preparation, while its under surface is supported by a needle held in the right hand. A still simpler method is to place a drop of the fluid in question on the lower side of the cover-glass and let it drop gently down upon the preparation. The fluid in which the section is placed should occupy the entire space between cover-glass and slide. If there is not enough fluid (*i. e.*, if numerous air bubbles appear under the cover-glass) place another drop of fluid at the edge of the cover-glass with the end of a glass rod. If there is now too much fluid (and therein the beginner especially is apt to err), that which is pressed out from under the edge of the cover-glass must be removed with filter paper. The upper surface of the cover-glass must always be dry. Small air bubbles under the cover-glass are removed by carefully raising and lowering it several times with a needle (see, further, p. 40).

Ad 1. One should never neglect to observe unstained, as well as stained, sections in water or normal salt solution (p. 19), for by this means many peculiarities of structure are sharply brought out, while they are likely to escape observation under the clearing influence of glycerine or of damar-varnish. Specimens examined in water or in salt solution cannot be kept long.

Ad. 2. Preparations examined in glycerine can be preserved. To prevent the shifting of the cover glass it may be fixed with cover-glass cement (see p. 22). Before this can be done the edge of the cover-glass must be perfectly dry, for the cement adheres only to the dry surface of the glass. The slide may be dried around the cover-glass by first removing the superfluous glycerine with filter paper, and then by carefully wiping with a cloth moistened in 90

* Examinations with low power, without the cover-glass, are designed only for the most superficial orientation, *e. g.*, to see whether a specimen has been sufficiently teased. In all other cases the cover-glass is indispensable.

Many a *good* specimen, if one neglects to cover it, appears useless. Examinations with high-power objectives, without a cover-glass, are, in general, not allowable.

per cent. alcohol and held over the end of the finger, taking care not to touch the cover-glass. Next heat a glass rod and dip it into the hard cement,* place a drop of the cement on each corner of the cover-glass, and trace a complete frame about the glass in such a way that the cement covers as well the cover-glass as the slide to an extension of 1 to 3 mm. Lastly, smooth the surface of the frame with the reheated rod.

Specimens preserved in glycerine often become transparent only after the second or third day. Hæmatoxylin and other stains fade after a short time; picrocarmine and carmine, on the other hand, are lasting in this fluid.

Ad. 3. The mounting of objects in *damar-varnish* is the usual preserving method. Damar-varnish has an advantage over glycerine in that it preserves the colors of the specimens, but has also the disadvantage of clearing the preparation much more than the dilute glycerine, thereby rendering many fine structural details entirely invisible.

The sections placed in water or alcohol of 90 or less per cent. cannot, without farther special treatment, be placed in damar-varnish. They must first be dehydrated. For this purpose the sections are placed by means of a needle (very thin sections with the section-lifter) into a covered watch-glass with 5 c.c. absolute alcohol. In doing this the least possible water should adhere to the sections. If a spatula is used, remove the water from it with filter-paper. If the section is transferred with a needle the water can be removed at the same time by lightly touching the section with filter paper. They stay in absolute alcohol two minutes (thin sections)—ten minutes (thick sections) or longer. † Then the sections, free as possible from alcohol, are transferred for clearing into a watch-glass with 3 c.c. bergamot oil. ‡ Place the glass on black paper so as to observe better the growing transparency of the sections. Avoid breathing into the watch-glass, for an immediate clouding of the bergamot oil takes place. If portions of the section, after two to three minutes, have not become transparent (such spots appear cloudy in direct light, dark brown in transmitted light), this indicates that the section was not sufficiently dehydrated, and hence that it must be put back in absolute alcohol. After being completely cleared the section is transferred to the dry slide and the superfluous oil|| carefully removed

* The glass rods crack very easily by heating; still, they are preferable to metal rods, which cool off too quickly. The cracking can be somewhat prevented by heating the rod to a red heat, meanwhile turning it constantly, for only rods which have been suddenly heated crack when dipped into the cement.

† For beginners may be recommended to place the sections taken from water in 5 c.c. 90 per cent. before placing them in the same amount of absolute alcohol.

‡ Fine sections may be transferred directly from absolute alcohol to the slide, where, after removing the superfluous alcohol with filter paper, a drop of bergamot oil is to be added. At first the oil will always retire from the section and must be brought back with a needle. After the completion of the clearing process, which one can ascertain with the low power, the excess of oil is wiped off and a cover-glass with damar-varnish added. When examining uncovered sections lying in oil, both sections and oil may become cloudy as a result of breathing upon them. In this case let the clouded oil run off and replace it with a drop of new oil.

|| The oil used for clearing in the watch-glass can be turned back into the bottle.

with filter-paper or with a piece of linen stretched over the index finger.* Then the cover-glass with a drop of damar-varnish adhering to its under surface is placed upon the specimen. If several sections are to be placed under one cover-glass, arrange them close together with a needle, then spread with a glass-rod an evenly thin coat of the varnish on the under side of the cover-glass and drop it on the sections. Large air bubbles are driven out by addition of a small drop of damar-varnish at the edge of the cover-glass. Within a day the bubbles disappear. The small bubbles disappear spontaneously, and can for this reason be neglected.

Beginners not seldom find that the varnish becomes cloudy and finally renders the preparation partially or entirely untransparent. The reason for this is that the sections were not entirely dehydrated. In the case of slight clouding, which under the microscope is seen to be due to minute drops of water, it is often sufficient to warm the slide a little. If the specimen is very cloudy the entire slide must be laid for one-half hour in turpentine. Then remove the cover-glass carefully, lay the section for two minutes in turpentine to loosen the adhering varnish, and, finally, to complete the dehydration, place the preparation in 4 c.c. absolute alcohol, which is to be changed after five minutes.

Since the damar-varnish dries very slowly the slides should not stand on edge but be in a horizontal position.

The series of processes, which a fresh specimen has to undergo before it may be preserved as a stained specimen, requires considerable time. If, *e. g.*, these directions are given in the Special Technique, "Preserve in Müller's fluid fourteen days, harden in gradually strengthened alcohol, color the section in carmine and hæmatoxylin, mount in damar-varnish," then the proceeding is as follows:—

The fresh specimen, about 1 c.c. in size, is put in 100 c.c.† *Müller's fluid*, which is changed as soon as it becomes cloudy, usually in one hour (§ 4, General Rules). In twenty-four hours the fluid is renewed, and in this the objects then remain for fourteen days.

At the expiration of this time—

Wash in (if possible running) water from one to four hours; then

Place in 20 c.c. distilled water fifteen minutes; next

Place in 50 c.c. 70 per cent. alcohol twenty-four hours, in the dark (see p. 29). Next

Place in 50 c.c. 90 per cent. alcohol twenty-four hours, and then transfer to fresh 90 per cent. alcohol.

The specimen thus fixed and hardened can be cut at any time. The sections are transferred from the alcohol (p. 30) to—‡

* The oil may be most easily and successfully removed by incising the slide as one wipes it.

† The quantities of the different fluids are reckoned only for this *one* piece, 1 c.c. in size. For several pieces or larger ones, more preserving and hardening fluid must of course be used.

‡ The following quantities are reckoned for 3 to 6 sections. With more sections the amount of the different fluids (of absolute alcohol especially) is to be increased.

- 20 c.c. weak carmine solution for twenty-four hours ; then to
- 5 c.c. distilled water for about ten minutes. Next into
- 5 c.c. hæmatoxylin for about five minutes,
- 30 c.c. distilled water for from ten to one hundred and twenty minutes,
- 5 c.c. absolute alcohol for ten minutes,
- 3 c.c. bergamot oil for two minutes, and finally mounted in damar varnish.

§ 11. EXAMINATION OF FRESH OBJECTS.

I have placed this method last because it is the most difficult, and presupposes a somewhat practiced eye. Experience is gained most easily by examination of specimens already *prepared* (hardened, colored, etc.). If one has once clearly seen and studied peculiarities of structure, then it is not so difficult to recognize them even in fresh specimens, though most of the details leave much to be desired in point of clearness. The following directions are to be observed :—

Slides and cover-glasses must not be oily. They should be cleaned with alcohol and dried with a clean cloth. Then put a drop of 0.75 per cent. salt solution (p. 19) on the slide, and in it place a small piece of the object to be examined, and cover with a cover-glass. The slightest pressure must be carefully avoided. In the case of very delicate objects (see Special Technique) support the cover-glass on two narrow strips of paper placed one on either side of the object. If the specimen requires no further treatment surround the cover-glass with paraffin to prevent evaporation. To do this melt a small piece of paraffin on the blade of an old scalpel and let it flow from this on the edge of the cover-glass, not from the tip but from the edge of the scalpel. In most cases the effect of certain reagents (acetic acid, potash-lye, stains) on fresh objects is studied directly under the microscope. It is then necessary to remove a part of the medium in which the specimen lies (in salt solution in the present instance), and to replace it with another fluid. For this purpose, first place a drop, *e. g.*, of picocarmine, by means of a glass rod, at the right edge of the cover-glass. Should the drop not touch the edge of the cover-glass, do not incline the slide, but lead it with a needle to the desired position. One now may observe that a little of the stain mixes with the salt solution, but does not spread out under the glass. To make the latter possible, place some filter-paper at the left edge of the cover-glass* and at once the picocarmine will diffuse under the entire surface of the cover-glass.† Now remove the filter-paper and let the stain act. When the staining is completed—this is to be ascertained under the microscope—place at the right edge of the cover-glass a drop of, *e. g.*, diluted glycerine, to which is added (in the case of picocarmine staining) as

* I cut a piece 4 cm. long, 2 cm. broad, fold it across, and place the paper roof thus formed on the slide in such a way that one smaller, perfectly straight edge touches the left edge of the cover-glass.

† When the first drop has penetrated place 2 to 3 additional drops at the right edge of the glass.

much acetic acid as will drop from a steel needle dipped into the acid (hence a very minute drop), while the filter paper is again applied to the left edge of the cover-glass. In this way a whole series of fluids may be passed through beneath the cover-glass and their action on the tissues be observed. Certain fluids, *e. g.*, picrocarmine, must remain very long in contact with specimens previously fixed in osmic acid. In this case evaporation may be prevented by placing the preparations in a *moist chamber*. To construct the latter one needs a porcelain plate and a small glass bell at least 9 cm. in diameter. Into the plate pour water 2 cm. deep. Then place in the middle a small glass dish or a cork plate standing on four wooden feet. On this the slides with the preparations may be laid and the whole covered with the glass bell, the free edge of which stands in the water.

§ 12. STORING OF PERMANENT SPECIMENS.

The mounted specimens must be labeled at once. Cardboard labels 1 to 2 mm. thick, stuck to the slide with fish-glue ("isinglass") are preferable to the mucilaged paper ones, for such mounted slides can be placed upon one another without pressing the specimens. The labels should be as large as possible (2 cm. square for slides of English form) and should have designated upon them the name of the organ, of the animal, and a brief indication of the methods used. For boxes and cases in which to keep the specimens, only those in which the slides lie horizontally should be chosen, and not those in which they stand on edge.*

* The best and cheapest cases are kept by Th. Schroeter, Leipzig, Windmühlenstr., No. 46. I recommend for box form, *pattern O* (for about 300 slides), price 2 *M.* (50 cents); for tray form, *P*, with flat covers for 10 to 20 slides (according to size), price 45 Pfennige (about 12 cents). The tray form have the great advantage of allowing all the specimens to be seen at once.

III. MANAGEMENT OF THE MICROSCOPE.

According to the position taken in the introduction, an exhaustive description of the optical and mechanical parts of the microscope cannot be entered upon here. Fig. 1 will recall to the reader the usual names of the several parts of the microscope.

The first requisite in the use of the microscope is perfect cleanliness of all its parts (see also p. 17). The surfaces of the mirror, objectives, and oculars should not be touched with the fingers. After the ocular has been placed in the upper end of the draw-tube, and a low-power objective screwed on the lower end of the tube (or on the revolver, if used), the field of view of the microscope should be illuminated with light reflected from a suitable source by the concave mirror placed below the stage. This is best accomplished by moving the mirror tentatively in all directions (with the diaphragm widely open, and the front lens of the objective about 1 cm. above the level of the stage) till the eye, looking simultaneously through the eye-piece into the microscope, sees the field of view brightly and uniformly illuminated.* Only the *concave* mirror should be used with the ordinary objectives recommended.

The best light for microscopic work is that obtained from a white cloud, or from a white window-blind illuminated by the sun. Of less value, but still of use, is the blue sky. Direct sunlight is to be avoided. Working in the evening with artificial illumination, light from the inner surface of a white lamp-shade (not directly from the flame) should be used. A plate of blue-glass placed before the mirror renders the artificial light more agreeable to the eyes, without blurring the outlines of the picture in a serious degree. It is obvious that the microscopist should not sit in direct sunlight. The instrument should be placed about a meter from the window.

Now the examination may begin. Always examine *first with the low, then with the high power*. Avoid especially the use of *strong oculars*, which narrow and darken the field of view, and thus make the examination much more difficult.†

* The rays of light reflected from the mirror in this position pass perpendicularly through the object on the stage. This is called *central illumination*. For distinguishing slight differences of level between adjacent parts of an object it is of advantage to use *oblique or lateral illumination*, to obtain which the mirror is moved to the side so that the rays reflected from it strike the object obliquely. When lateral illumination is used the whole diaphragm should be taken away, so that the opening in the stage shall be as large as possible.

† Nearly all the preparations used for the illustration of this book were examined and drawn with weak oculars.

The increased magnification obtained by drawing out the draw-tube is seldom necessary. With low powers the aperture in the diaphragm should gen-

FIG. 1.—LEITZ MICROSCOPE. STAND II ($\frac{1}{2}$ natural size).

erally be large, with high powers small. In focusing the object, the coarse adjustment by rack and pinion is used first. With its aid the objective is first

placed at a distance from the object greater than its focal length, and then with the eye applied to the ocular the tube is gradually lowered, till the outlines of the preparation appear in the field of view. The image is then brought into distinct view by means of the fine adjustment or micrometer screw. In doing this the left hand should hold the slide, while the right remains on the micrometer screw. In the examination of the preparation the tube should be slightly raised and lowered by the micrometer screw, because only the points lying in *one* plane of the preparation can be in focus and distinctly seen at one time. In using the microscope the habit should be formed of keeping both eyes open.

One should never neglect to examine the preparations also with a hand-lens. For this purpose the oculars (*e. g.*, Leitz, Oc. III) can be used. The mounted specimen is held with the cover-glass side toward the light. The upper lens of the ocular is placed directly against the slide, the eye applied to the lower or back-lens.

DRAWING.

Drawing is a very valuable aid in microscopical work. The power of observation is made considerably keener, and many details, which would be otherwise completely overlooked, are discovered while the sketch is in progress. Even the most attentive examination cannot replace the advantage which drawing yields. Those who have little practice in drawing should nevertheless try to sketch the preparations under both low and high power. For this purpose the drawing paper should be on a level with the stage, the left eye applied to the microscope, the right directed to the paper and the pencil-point. At first this is rather difficult, but a little practice will soon give the necessary facility.

MEASUREMENT.

For this purpose an ocular-micrometer and stage-micrometer are used.*

The latter is laid on the stage and focused, and the number of divisions of the ocular-micrometer which corresponds with one part of the stage-micrometer is determined.†

As the real size of the divisions on the stage-micrometer is known, it is easy to determine the size of the object, which, with a given stage- and ocular-micrometer, corresponds with one or more divisions of the ocular-micrometer. The following example may make the method clear: With Leitz Objective 3,

* Ocular-micrometers are in some cases (Leitz) made to simply rest upon the diaphragm inside the ocular; or in other cases (Seibert) to be inserted through a lateral opening; in others, again (Zeiss), specially constructed oculars for measuring are provided for the microscope. The real size of the divisions of the ocular-micrometer need of course not be known. The stage-micrometer is a glass-slide on which a millimeter divided into 100 parts is engraved. Instead of this a second ocular-micrometer, which is usually divided only into 20ths of a millimeter, may be used. Measurements made with this are naturally not so accurate, but the error is so slight that it scarcely need be considered.

† Beginners often have trouble to focus the lines of the stage-micrometer. Weak or oblique illumination makes this more easy.

Ocular I, and draw-tube pushed in, 5 divisions of the ocular-micrometer correspond with 1 division of the stage-micrometer. A division of the stage-micrometer used $= \frac{1}{20}$ mm. Hence 5 divisions of the ocular-micrometer $= \frac{1}{20}$ (0.05 mm.), and 1 division of the ocular-micrometer $= 0.01$ mm. If, then, any microscopic object, *e. g.*, a striated muscle-fibre, the diameter of which it is required to know, occupies 4 divisions, the fibre is 0.04 mm. broad.

It is often difficult, especially with low magnification, to count the fine divisions of the ocular-micrometer. This can be done more easily by taking advantage of the longer lines, marking every fifth or tenth division. For instance, with Leitz Objective 3, Ocular I, and the draw-tube drawn out, 40 divisions of the ocular-micrometer correspond with 5 divisions of the stage-micrometer. Therefore, 40 divisions $= \frac{5}{20}$ mm. $= 0.25$ mm., and one division of the ocular-micrometer with this magnification $= 0.00625$ mm., 2 divisions $= 0.0125$ mm., and so on.

With Leitz Objective 7, Ocular I, and draw-tube pushed in, 30 divisions of the ocular-micrometer correspond with 1 division of the stage-micrometer; 30 divisions $= 0.05$ mm., 1 division $= 0.0017$ mm., or 7 μ . Finally, with Leitz Objective 7, Ocular I, and draw-tube drawn out, 40 divisions of the ocular-micrometer $= 1$ division of the stage-micrometer. Therefore, 40 divisions $= 0.05$ mm., 1 division $= 0.00125$ mm., or 12 μ .

It is advisable, if one has many microscopic measurements to make, to prepare a table for each magnification made use of, in which the real values of 1, 2, 3 20, 30, 40 100 scale divisions of the ocular-micrometer are given. It must be emphasized that the examples above given by no means apply to all the microscopes made by Leitz. The values must be specially determined for every instrument by the above given method.

In conclusion, the microscopist is exhorted to patience—great patience! If his preparations fail, let him seek the cause in himself, not in the deficiency of the methods recommended, for I have often tested them. He who cannot accustom himself to follow out conscientiously the directions given,* who takes hold of delicate objects with all five fingers, who contaminates his reagents one with another, who puts his specimens in their fixing fluids in the sun or lets them dry up, is not justified in expecting good results from his careless work.

* The lengths of time given for staining, dehydrating, etc., have only an approximate value. They vary within considerable limits in accordance with the thickness of the sections, the concentration of the solutions, etc. Experience will soon teach the microscopist to hit the proper time.

PART II.

MICROSCOPICAL ANATOMY.

The animal body is composed of cells which are all produced from a single cell by repeated division. At the beginning of development the cells are of like form, all being spherical, and none is furnished with special characteristics to distinguish it from its companions. The cells are still *undifferentiated*. In the course of development, the cells arrange themselves in flat superposed layers, the so-called *germ-layers*. With the separation in germ-layers, and with the formation of organs from these, the cells cease to resemble one another,—they become *differentiated*. In general, the cells which have been differentiated in the same direction are united into webs or complexes, and form a *tissue*. *A tissue, therefore, is a complex of similarly differentiated cells.* We distinguish four principal tissues: 1. *Epithelial tissue*. 2. *Connective tissue*. 3. *Muscular tissue*. 4. *Nervous tissue*. So long as these tissues are still young, they are composed only of similar elements, of cells; in the course of development, however, this condition is changed in a two-fold manner. First, the cells produce special substances, which, being deposited between them, are called *intercellular substances*. By this process the character of the tissue, however, is not essentially altered. The definition of tissue given above need only be so far extended that we call a tissue a *complex of similarly differentiated cells and their derivatives*. More radical is the second change, consisting in a penetration of tissues of one kind by those of another. The extent of this change varies greatly in different cases. It is least marked in the case of the epithelial tissues, more so in the connective tissues. Muscular and nervous tissues in their developed forms are mixed with other tissues to such a degree that even though in the differentiated elements muscle and nerve predominate, we can hardly, according to the definition given above, speak of the structures as “tissues.” *

The tissues are, therefore, not equivalent among themselves as regards differentiation. Epithelial tissues and connective tissues stand in the lowest rank; both these, differing from one another in form and function, occur in plants as well as animals; we can, therefore, class them as *vegetative tissues*. On a higher level, both morphologically and physiologically stand the muscular

* For this reason the proposition has been made to omit a division of tissues and to distinguish only elements and organs.

and nervous tissues, which, being found only in the animal body, are called *animal tissues*.

When different tissues are united in a structure of definite form and definite function, they constitute an *organ*. Our task, therefore, consists in: 1, the study of the cells and of the tissues, and, 2, in the study of the organs. The investigation of cells and of tissues is the object of *histology*. Histology is a part of general anatomy, which, because of the instrument most used in its study, is called *microscopic anatomy*. The investigation of organs, also, so far as it can be done with the aid of the microscope, is the task of microscopic anatomy.

I. HISTOLOGY.

(*Microscopic Anatomy of Cells and Tissues*.)

A. CELLS.

A cell, *cellula*, is a structural element which, under certain conditions, is able to nourish itself, to grow and to multiply. In virtue of these properties the cell is called an *elementary organism*.

The essential parts of a cell are: 1. The *protoplasm*, or cell-substance, a soft, semi-fluid substance of alkaline reaction, insoluble in water, highly distensible, consisting principally of albuminous substances, much water and salts, and containing a special nitrogenous proteid, *plastin*. In the protoplasm small granules, *microsomes*, occur in variable numbers, and when numerous, give to the protoplasm a darker appearance. They are irregularly distributed; are absent in the superficial layer (exoplasm), which is somewhat denser and perhaps possesses a special function. With the aid of very high magnifying power it will be seen that protoplasm possesses structure: a reticulum, *spongioplasm*, which is embedded in an amorphous ground-substance, *hyaloplasm* (Flemming).* 2. The *nucleus*, a sharply defined, usually vesicular body lying in the middle of the cell, and consisting of several *proteid* substances, *chromatin* (nuclein), and *pyrenin* (paranuclein), besides *linin*, the *nuclear fluid* (matrix), and *amphipyrenin*. Both chromatin and pyrenin, by their affinity for stains, are distinguished from the other three so-called achromatin substances, but differ chemically from one another. For example, on the addition of distilled water the structures composed of chromatin disappear, while those com-

*The theories concerning the structure of protoplasm are by no means agreed. According to Fromann, Leydig, and others, protoplasm is a spongy structure, that is, it consists of a network whose meshes contain a fluid. According to Bütschli, the structure is froth-like, that is, it contains small spaces which do not communicate with each other. According to the much-disputed theory of Altmann, protoplasm is composed of granules (granula, bioplasts), connected by an indifferent substance, and these are the real elementary organisms.

posed of pyrenin remain intact. In the simplest case (in spermatozoa), the nucleus is a compact mass of chromatin, to which the pyrenin is attached, but usually it is composed of a network of fine linin threads and of coarser chromatin cords.* The chromatin cords are of different caliber, and exhibit, at intervals, isolated *enlargements*, which must not be confused with the nucleoli. Linin and chromatin form the *nuclear network*, whose interstices are occupied by one or more nucleoli (consisting of pyrenin) and the nuclear fluid. The nuclear membrane, not always present, is composed of amphipyrenin. Often a membrane is simulated by a superficial layer of chromatin. The nuclear network and nucleoli undergo important changes with the increasing age of the cell.

To the nucleus belongs the *centrosome*, a minute corpuscle from which fine threads extend to the chromatin cords and to the nuclear membrane. Because of its minuteness it can be seen only in particularly favorable objects (in the

FIG. 2 — DIAGRAM OF A CELL. Microsomes and spongioplasm are only partly drawn.

spermatocytes of *ascaris megaloccephala univalens*, in carcinoma cells), and then indistinctly, until it wanders from the nucleus into the protoplasm, which it does during the division of the cell. In the protoplasm the centrosome can be more readily seen, and seems to be able to remain there for a considerable period. There it was first discovered, and on this account was regarded, erroneously, as a constituent of the protoplasm (Fig. 3).

Most cells contain but one nucleus; only a few have several nuclei (some wandering cells, giant cells, and others). Non-nucleated cells (horny cells of the epidermis, colored blood-corpuscles of mammals) originally possess nuclei, but lose them in the course of development.

* In certain suitable preparations it may be seen that the chromatin cords are composed of rows of granules which lie in contact with threads of linin. This is shown in the upper half of the diagram (Fig. 2).

An unessential element of the cell is the *cell-membrane*, which is wanting in many cells, and when present, is either a transformation of the peripheral zone of the protoplasm or a secretory product of the latter. It appears as a thin, usually structureless, membrane. The protoplasm of cells may contain adventitious materials, pigment, glycogen, etc., and globules of fat, of aqueous and slimy fluids. The term paranucleus (*Nebenkern*) has been used to designate various structures, the significance of which is not yet determined. A paranucleus is often simulated by the remnants of degenerated cells which have been incorporated in a living cell. In other cases the paranucleus is confused with the centrosome.

FIG. 3.—CELL OF THE BONE-MARROW OF A RABBIT. $\times 1500$. The double centrosome lies in a clear area, the attraction-sphere.

Cells differ greatly in form. They may be: *spherical*, the typical form of all cells in the embryonal period, and in the adult, for example, resting leucocytes are spherical; *discoid*, e. g., the colored blood-corpuscles; *polyhedral*, e. g., the liver cells; *cylindrical* or *columnar*, e. g., the epithelium of the small intestine; *cubical*, e. g., the epithelium of the capsule of the crystalline lens; *flattened* (so-called squamous epithelium), e. g., the epithelial cells of the blood-vessels; *spindle-shaped*, e. g., many connective-tissue cells; *elongated into fibers*, e. g., smooth muscle-fibers; and *stellate*, e. g., many ganglion-cells. The form of the nucleus usually corresponds to the form of the cell. It is more or less oval in columnar, spindle, and stellate cells; rounded in spherical and cubical cells. Lobulated, so-called polymorphous, nuclei are

FIG. 4.—LEUCOCYTES OF A FROG. $\times 560$. Changes in form observed during ten minutes. Techn. No. 43.

found in leucocytes and in giant cells, and are a symptom of activity on the part of the cell, tending either to locomotion or change in form, or to increased metabolic energy.

The size of cells varies from forms microscopically small ($4\mu^*$) (colored blood-corpuscles) to macroscopic bodies (eggs of birds, amphibians). The size of the nucleus corresponds in general to that of the protoplasmic body. Only mature ova, despite their great dimensions, have minute nuclei.

* Λ μικρον, mikron = μ = 0.001 mm.

The *vital properties* of cells will be discussed only in so far as they can be studied by direct microscopic observation; other details must be sought in text-books of physiology. Accordingly, there will be considered here the phenomena of motion in cells, the reproduction of cells, and those microscopic processes which are associated with the secretory activity of cells.

The *phenomena of motion* occur in the form of amœboid* activity, of ciliary motion, and of contraction of certain fibers (muscle-fibers). The amœboid movement is the most important, and has been observed in nearly all the cells of the animal body. In especially favorable cases, *e. g.*, in leucocytes, the protoplasm of the cells throws out finer or coarser processes (pseudopodia), which by dividing and flowing together produce a great variety of forms. These processes may retract, or they may become fixed and draw the remainder of the cell-body after them, the result of which is locomotion, or the so-called "wandering" of cells. These wandering cells play an important part in the economy of the animal body. The processes can flow around and inclose foreign particles or small cells, an incident described as the feeding of the cell.† Amœboid movements ensue very slowly; in warm-blooded animals, only on artificial warming of the object. For ciliary motion and contraction see the Epithelial and the Muscular Tissues.

There is still another movement which is observed not only in the living but also in the dead cell. This is the so-called *molecular motion*, an oscillation of minute granules in the cell, the result of molecular currents in the fluid in which they are suspended. It may often be observed in the salivary corpuscles (see Lymph-follicles of the Tongue).

Reproduction and Multiplication of Cells.—Formerly, two kinds of cell formation were distinguished, spontaneous generation (*generatio æquivoca*) and generation by division. According to the theory of spontaneous generation, cells originate in a suitable fluid, *cytoblastema*. This view has been utterly abandoned. Only one kind of cell-generation is now recognized; that is, reproduction by *division* of preëxisting cells, "*Omnis cellula: cellula.*"‡

In the division of a cell, first the nucleus and then the protoplasm, divides into two usually equal parts. In this process a special grouping and rearranging of the nuclear substances take place according to definite laws. This mode of division is called *indirect division*, *mitosis*,|| *karyokinesis*. Its cycle is usually divided into three phases, as follows:—

* This movement is exhibited in its perfection by unicellular organisms named amœbæ,—hence the phrase "amœboid movement."

† Not to be confused with the nutrition of the cell, which is effected by a series of complicated chemical processes within the cells; diosmotic currents, imbibition, molecular pressure, etc.

‡ Likewise, a new nucleus can be formed only by the division of an existing nucleus. The theory of spontaneous generation of nuclei, according to which nuclei originate directly from the protoplasm and independently of existing nuclei, lacks convincing evidence.

|| *μήτος* = thread, because in this process threads are visible in the nucleus. There is a second mode of division, in which the nuclei divide simply by constriction, without a definite grouping of the nuclear substances. This is called *direct* or *amitotic division*. It is,

(1) **Prophase.**—The centrosome increases in size and migrates from the nucleus into the protoplasm. There it lies near the nuclear membrane, surrounded by a clear zone from which delicate threads radiate. The area occupied by these threads is called the *attraction-sphere*. The nucleus enlarges; the nuclear network becomes richer in chromatin, and the chromatin cords assume the form of tortuous segments, chromosomes,* transversely disposed to the longitudinal axis of the nucleus, and the number of which is constant for each animal species. The form of these segments is usually that of a V-shaped loop. The apices or closed ends of the loops are directed toward a common center, the *polar field*,—the area in which the centrosome is situated—their free ends toward the opposite pole of the cell. This arrangement of the segments is called the *close skein*. It is followed by a further thickening of

FIG. 5.—KARYOKINETIC FIGURES OBSERVED IN THE EPITHELIUM OF THE MOUTH CAVITY OF A SALAMANDER. The picture in the upper right-hand corner is from a section through a dividing egg of *Siredon pisciformis*. The centrosomes, also the first stages of the development of the spindle, cannot be seen by this magnification. $\times 560$. Techn. No. 16.

the segments and the formation of the *loose skein*, in which the loops are less tortuous, and some have their closed ends turned away from the polar field.

however, very probable that this kind of division in vertebrates has not the significance of a *physiological* multiplication of cells, but occurs only in those cells which are on the point of disintegrating, for very often the division of the protoplasm does not follow, so that only a multiplication of nuclei takes place. This frequently happens in leucocytes, also in epithelia, *e. g.*, in the superficial epithelial cells of the bladder of young animals.

* These segments are present also in many resting nuclei, but are not easy to distinguish because of the many lateral branches by which they anastomose with their fellows to form a network. When the process of division begins the lateral twigs are retracted, and consequently the segments become thicker and more conspicuous. In other nuclei the chromatin appears as a single filament, which subsequently divides into chromosomes.

Meanwhile the centrosome has undergone division into two, each surrounded by an attraction-sphere. The two centrosomes then move apart, and the interval between them is spanned by delicate fibrils, which form the *central spindle*. This soon disappears. The centrosomes continue to move apart along the nuclear membrane through an arc of 90 degrees. The threads extending from the centrosomes to the chromosomes persist. Toward the completion of the prophase the nuclear membrane vanishes and the nucleolus becomes invisible.

(2) **Metaphase.**—The centrosomes have reached diametrically opposite points,* and the threads extending from them to the chromosomes, and with which parts of the nuclear membrane may be associated, now appear in the figure of a spindle, the *nuclear spindle*. At each apex of the spindle is a centrosome surrounded by an attraction-sphere, which in this stage is also known as the “polar radiation.” The chromatin loops move to the equator of the spindle, in the future plane of division of the nucleus, and arrange themselves with their closed ends directed toward the axis of the spindle. Viewed from the apex of the spindle this grouping of the segments has the appearance of a star, *mother-star* (monaster).

During the formation of the mother-star, often earlier, in the first stages of the prophase, the chromatin loops split longitudinally, and each forms two “sister-loops.” Division of the nucleus exactly into halves now follows as a result of the contraction of the threads of the spindle, by which one sister-loop of a pair is drawn to one pole, the other to the opposite pole of the spindle. This is called *metakinesis*. In this stage the nuclear segments appear in the form of two *daughter-stars* (diaster).

(3) **Anaphase.**—These figures are soon obliterated. The lateral twigs of the chromosomes reappear, anastomose with one another, and reproduce the reticulum of the resting nucleus. Meanwhile the spindle has become invisible, and also the greater portion of the polar radiation, the nuclear membrane is reformed, the nucleus reabsorbs the nuclear fluid, swells, and becomes spherical, and the nucleolus reappears. At the same time the hitherto quiescent protoplasm begins to divide, a furrow appearing at the equator of the cell and deepening until the separation into two halves is accomplished.

In rare cases of mitotic division, especially in those of a pathologic nature, the nucleus divides simultaneously into more than two.

The duration of cell-division varies from a half hour (in man)† to five hours (in amphibians).

Special modifications of cell-division are the so-called *endogenous cell-formation* and *budding*. The former occurs in those cells which are enveloped

* The above description of the behavior of the centrosomes does not always hold good. For example, the centrosome in *Ascaris megalocephala univalens* divides within the nucleus, which elongates and extrudes a centrosome at each end. During their extrusion the nuclear spindle is formed. In succeeding events the processes are identical.

† The disappearance of the mitotic figures in the human cadaver is not complete until after an elapse of forty-eight hours.

in a firm capsule (eggs, cartilage cells), and the mode of division is precisely the same as that described above, only that all the descendants of the mother-cell remain inclosed in the common capsule. Gemmation or budding indicates a kind of unequal cell-division, in which protoplasmic processes of the cell are set free, and become independent cells (as in bone-marrow).

The young cells always resemble in character the mother-cells. Such a case as a connective-tissue cell arising from the division of an epithelial cell never occurs.

The Phenomena of Secretion.—See Secretory Activity of Epithelial Tissue.

The *length of life* of all cells is limited. The old elements disintegrate, new ones appear in their places. Formerly these phenomena were not distinguished from secretory processes, and the erroneous idea was entertained that the process of secretion terminated in the death of the cell. Dying cells are characterized by decrease in the volume of both nucleus and protoplasm. The latter often presents a notched edge or stains deeply, while the chromatin substance of the nucleus appears either shrunken or in the form of irregular fragments that react alike to stains. Vacuolization of the protoplasm or the nucleus is another symptom of degeneration.

The *growth* of cells concerns preëminently the protoplasm and only exceptionally takes place equally in all directions, in which case the original form of the cell is retained (*e. g.*, egg-cell); as a rule an unequal growth takes place. As a result of unequal growth the original form is altered; the cell becomes elongated, or flattened, or branched, etc. The majority of cells are soft and susceptible to change in form from mechanical influences, as, for example, the columnar epithelial cells in the empty bladder, which are flattened in the distended organ.

Secretory Products of Cells.—The secreted materials are either wholly removed from the cells (as most glandular secretions) or they harden and remain on the surface of the cells. To the latter belong certain *intercellular substances*, many of which are secretions of the cells; others are produced by change in the peripheral layers of the cell-protoplasm, still others, by a complete transformation of the cells themselves (?). It is very difficult to decide whether individual intercellular substances were formed by one process or another. Many points in this matter are still sharply disputed.

The intercellular substance occurs either in small amount, as a structureless *cement-substance*, found between epithelial cells, connective-tissue cells, smooth muscle-fiber, etc.; or in large amounts exceeding the mass of the cells, and is then called *matrix* or *ground-substance*. The matrix is either formless (homogeneous) or formed. In the latter case it consists for the most part of fibers or granules of different kinds. The remnants of formless substance found between the fibers or granules are also called cement-substance.

B. TISSUES.

I. THE EPITHELIAL TISSUES.

The elements of epithelial tissue, the *epithelial cells*, are definitely outlined cells consisting of protoplasm and a nucleus. A cell-membrane is frequently absent, or is represented by a condensation of the peripheral zone of protoplasm. The majority of epithelial cells are soft and plastic, and yield readily to the pressure of neighboring cells, the result of which is great diversity of outline. In general two principal forms can be distinguished: the *flattened or squamous* and the *cylindrical or columnar* (better, prismatic). These extremes are united by numerous transitional forms.

The squamous epithelial cells are seldom regular in form, excepting the pigmented epithelium of the retina, which consists of tolerably regular hexagonal cells. With this exception the outlines are usually very irregular.

FIG. 6.—EPITHELIAL CELLS OF RABBIT, ISOLATED. $\times 560$. 1. Squamous cells (mucous membrane of mouth), Techn. No. 85. 2. Columnar cells (corneal epithelium). 3. Columnar cells, with cuticular border, *s* (intestinal epithelium). 4. Ciliated cells; *h*, cilia (bronchial epithelium).

The cylindrical epithelial cells, *cylinder or columnar cells*, seen from the side, are elongated elements whose height considerably exceeds their breadth, and, seen from above, appear hexagonal; they are therefore in reality prismatic columns. Cells as high as they are broad are called cubical epithelial cells.

[Since any form of epithelium viewed from the free surface may present a mosaic, the term pavement is not distinctive.]

Many columnar cells have a sometimes homogeneous, sometimes striated, border on their free upper surface, the *cuticular zone* (Fig. 6, 3 *s*).

The striae are the optical expression of minute rods, occasionally distinctly seen even with medium magnification (Fig. 9 *c*); they differ greatly in length, and answer to processes of the protoplasm that penetrate the homogeneous zone. To the same category belong the striations seen in the basal half of the cells lining the smaller ducts of the salivary glands and some of the tubules of the kidney. The latter are distinguished by their greater delicacy and their relation to secretory activity (they have been seen only in secreting cells).

Other columnar cells are beset with delicate filamentous processes (cilia) on their free surface, which during life are in constant active vibration to and fro in a definite direction. These are called *ciliated cells*.

The specially differentiated neuro-epithelial cells will be described in connection with the organs of special sense.

The epithelial cells are united by means of a very small amount of cement-substance, and present either smooth surfaces of contact to one another or uneven surfaces and an interlocking of variously-shaped processes,—the processes being pressure-effects—that is, the result of the mutual pressure of contiguous cells.

The minute spines and ridges that beset the surface of certain epithelial cells have been included among these processes. But they are protoplasmic connecting filaments that penetrate the cement-substance and establish a close internal union between neighboring cells. Cells furnished with such spines and ridges are called *prickle-cells*, and the processes are designated *intercellular bridges*.

Continuous layers of epithelial cells, covering outer and inner surfaces of the body, are called “epithelia.” The epithelia are sometimes composed of a

FIG. 7.—FROM A VERTICAL SECTION OF THE STRATUM MUCOSUM OF THE EPIDERMIS. $\times 360$. Seven prickle-cells united by intercellular bridges. Techn. like No. 83.

FIG. 8.—PIGMENTED EPITHELIUM OF RETINA OF MAN. Viewed from the surface. $\times 560$. Techn. No. 170 b.

FIG. 9.—SIMPLE COLUMNAR EPITHELIUM OF INTESTINE OF MAN. $\times 560$. c. Striated cuticular border. z. Columnar cell. tp. Tunica propria. Techn. like No. 102.

single stratum, sometimes of several strata, and accordingly the following varieties are distinguished :—

1. *Simple squamous epithelium* : in the outer layer of the retina, in the alveoli of the lungs, the rete vasculosum Halleri, the membranous labyrinth, the choroid plexuses and parts of the ventricles of the brain, the posterior surface of the anterior capsule of the lens, in parts of the ducts of glands, and in the Malpighian body and descending limb of Henle's loop in the kidney ; also in the peritoneum, the articular cavities, the tendon-sheaths, the bursæ, the blood- and lymph-vessels. The five last mentioned epithelia are also called *endothelia*—their elements *endothelial cells*.

2. *Simple columnar epithelium*.: in the intestinal canal and in the ducts of many glands.

3. *Simple ciliated epithelium* : in the smallest bronchi, in the uterus and oviducts, in the accessory spaces of the nasal fossæ, in the central canal of the spinal cord.

4. *Stratified squamous epithelium* : not all the elements of which are flattened cells. The lowermost stratum is composed of columnar cells. Superposed on this are several strata of variously-shaped cells, mainly irregular

polygonal prickles-cells, over which lie successive strata of cells that, as they approach the surface, become gradually thinner and flatter (Fig. 10). The stratified pavement epithelium occurs in the mouth and pharynx, in the oesophagus, on the vocal cords, on the cornea, in the vagina, and in the female urethra. The epidermis also consists of a stratified pavement epithelium, but is characterized by the cornification of the cells of the superficial strata, which are transformed into horny scales without nuclei. Cornified cells are found also on the hairs and nails, but in these situations are nucleated.

5. *Stratified columnar epithelium*, in man, is found only on the conjunctiva palpebrarum, in the main excretory ducts of certain glands, and in a portion of the male urethra. The arrangement of the strata is similar to that of—

6. *Stratified ciliated epithelium*, in which only the most superficial cells are columnar and bear cilia, while in the deepest layers the elements are mainly spherical, and in the middle layers, spindle-shaped (Fig. 11). Stratified ciliated

FIG. 10.—STRATIFIED SQUAMOUS EPITHELIUM (LARYNX OF MAN). $\times 240$. 1. Columnar cells. 2. Prickle-cells. 3. Squamous cells. Techn. No. 122.

FIG. 11.—STRATIFIED CILIATED EPITHELIUM. $\times 560$. From the respiratory nasal mucous membrane of man. 1. Oval cells. 2. Spindle-shaped cells. 3. Columnar cells. Techn. No. 191.

epithelium is found in the larynx, in the trachea, in the larger bronchi, in the nasal cavity, in the upper part of the pharynx, in the Eustachian tube, and in the epididymis.

The epithelium has no blood- and lymph-vessels, but nerves are found in some situations, for example, in the epithelium of the skin and of many mucous membranes.

Secretory Activity of Epithelial Tissue.—Many epithelial cells are capable of secreting and discharging certain substances which are not used for the growth and development of the tissue. Such cells are called *glandular cells*. The secreted substances are either used in the body (secretions) or, those of no further use, removed from the body (excretions). The performance of the processes of elaboration and discharge of secretions (or excretions) is manifested by certain changes in the appearance of the form and contents

of glandular cells, and which indicate conditions of rest and activity. In many, *e. g.*, serous glandular cells, the differences are confined (barring certain phenomena in the nucleus) to decrease in volume and a dark appearance of cells empty of secretion, and to increase in volume and a clear appearance of those filled with secretion. In other gland-cells, *e. g.*, in many mucous glands, the process of secretion can be traced more accurately. Granular protoplasmic contents and a usually oval, nearly centrally situated nucleus indicate a condition of exhaustion. The elaboration of secretion begins at the free surface of the cell, that directed toward the lumen of the gland, and manifests itself by the transformation of the granular protoplasm into a clear mass (*b, s*), more or less sharply defined against the still unaltered protoplasm (*b, p*). As secretion progresses, more and more of the protoplasm is transformed, and the nucleus and remnant of unaltered protoplasm are pushed to the bottom of the cell. As a consequence of this gradual compression, the nucleus is rounded or even flattened. The volume of the secreting cell when filled is considerably enlarged. Finally, the cell-wall bursts at its free surface. The secretion gradually escapes, and simultaneously the protoplasm is regenerated, the nucleus moves upward to its original position, and the cell, diminished in size, is restored to its previous condition and appearance. The

FIG. 12.—SECRETING EPITHELIAL CELLS. From a thin section of mucous membrane of the stomach of man. $\times 560$. *p*, Protoplasm. *s*, Secretion. *a*, Two cells empty of secretion; the cell between them shows beginning mucoid metamorphosis. *e*, The cell on the right is discharging its contents, its upper free wall having ruptured; the granular protoplasm has increased, and the nucleus has become round again. Techn. No. 102.

majority of glandular cells do not degenerate in the act of secreting, but are able to repeat the process again and again. The cells of the sebaceous glands furnish an exception, for, like the goblet-cells, their secretion is formed by the disintegration of cells.* In the case of the latter the processes of elaboration and expulsion of the secretion occur simultaneously; at first the secretion is produced more rapidly than it is discharged, and it accumulates in the cell, but at the last expulsion exceeds production, the cell gradually empties itself completely, and dies (Fig. 13).

The glandular cells lie isolated between other epithelial cells† or are united in groups and form glandular tissue.

* The testicle and ovary furnish a peculiar instance, the gland cells of which, after secretion, undergo further development.

† They are then called unicellular glands, and are very common among invertebrates, but appear also in man as goblet-cells.

Supplement.—The Glands.—The glands are composed almost exclusively of epithelium (glandular), and therefore, although they are organs, they may be described with the epithelial tissues. Connective tissue and blood-vessels are present, and though very important physiologically, are less so morphologically.

The glands occur in two principal forms: as cylindrical tubules or as rounded saccules.

The *tubular* glands occur either singly or combined into groups; therefore they are divided into—

FIG. 14.—CRYPT OF LIEBERKÜHN FROM A SECTION OF THE LARGE INTESTINE OF MAN. $\times 165$. The secretion formed in the goblet-cells is dark in color. In zone 1 the goblet-cells show the beginning of secretion. That a part of the secretion is already given off here is evident from the presence of secretion in the form of drops in the lumen of the crypt. 2, Goblet-cells with much secretion. 3, Cells containing a small amount of secretion. 4, Degenerating goblet-cells, some of which still contain remnants of secretion. Techn. 10.

1. *Simple tubular glands*, which have the form of simple or branched tubules (Fig. 14); the latter may be called a "duct-system."*

* The true form of such glands can be recognized only on the most exact investigation, because the branched tubules are twisted about one another or coiled in a dense convolution. They were formerly called "racemose glands."

2. *Compound tubular glands*, which consist of a large and variable number of "duct-systems" (Fig. 14).

The same division is applicable to *alveolar glands*. They occur as—

FIG. 14.—DIAGRAM OF THE DIFFERENT GLAND-FORMS. a. Excretory duct.

1. *Simple saccular (alveolar) glands*, which, similarly, are simple or branched saccules having an excretory duct; the latter are termed "alveolar system."

2. *Compound saccular (alveolar) glands*, which consist of a combination of several "alveolar systems" (Fig. 14).

Simple unbranched tubular glands: the peptic or fundus glands, the sweat-glands, and the glands of Lieberkühn.

Simple branched tubular glands: the pyloric glands, the glands of Brunner, the smallest glands of the oral cavity, the glands of the tongue, and the glands of the uterus.

Compound tubular glands: the mammary, the salivary, the lacrymal and the larger mucous glands,* the kidneys, the glands of Cowper, the prostatic glands, the thyroid gland, the testicle, and the liver. The branches in the last two anastomose and form networks, and hence are also called "reticular glands."

Simple unbranched saccular glands: the smallest sebaceous glands and the follicles of the ovary.

Simple branched saccular glands: the larger sebaceous glands and the Meibomian glands.

Compound saccular glands: the lungs.

In the majority of glands, especially in those visible to the naked eye, a sheath is formed by the surrounding connective tissue, which sends septa into the gland and divides it into compartments of varying size, the *gland lobules*. The septa carry the larger blood-vessels and nerves. The glands may secrete throughout their entire extent, but usually only that part lying near the blind end (*gland follicle*) is specialized for this purpose, while the part forming the connection with the surface serves for the conveyance of the secretion, and is called *excretory duct*.

Glands without excretory ducts are the *thyroid body* and the *ovary*. The former has an excretory duct in the embryonic period, which disappears, however, in the course of development. The gland follicles of the ovary, in the embryonic period, are in communication with the superficial epithelium; these connections, which might be called excretory ducts, disappear, and the expulsion of the products formed in the ovary (the ova) takes place by the bursting of the follicles. The ovary is a *dehiscent* gland.

All gland follicles are composed of a usually simple layer of gland cells, which bound the lumen of the gland and are in turn surrounded by a special modification of the connective tissue, a *membrana propria* or *basement membrane* (see p. 67). Occasionally, instead of this, the gland tubules are embraced by stellate, nucleated cells ("basket-cells"). On the outer side of the

* The cross-sections of the coiled and closely-packed branching tubules of the last three glands were for a long time regarded as vesicular evaginations of the terminal ends of the tubules, and were named acini. Such evaginations (except in a few isolated parts of the sublingual gland do not really occur; the diameter of the lumen is not larger here than in other portions of the tubules. On the other hand, a thickening of the wall of terminal parts of tubules, by taller cells, is not uncommon in some tubular glands, e. g., in the parotid and the pancreas. Such thickenings, however, must not be called "acini," since we understand by acinus an evagination, a distention of the lumen. To avoid misunderstanding, the term "acinus" was dropped and that of "alveolus" selected for glands of the saccular form. Likewise the much-used term "acinous" or "racemose" (alveolar) has been discarded, because the cross-sections of tubular glands also exhibit a "racemose" appearance.

basement membrane the blood-vessels are situated (Fig. 15). The gland-cells are inserted between the blood-vessels and the lumen of the gland, and on the one side receive from the blood-vessels (or the surrounding lymph-vessels) the crude materials necessary to secretion, and on the other side give off the elaborated materials as secretion.

In some glands, *e. g.*, the fundus glands of the stomach, the cell discharges the secretion not only on the free surface but on all sides. The secretion then passes into a network of canaliculi that envelopes the cell and communicates with the lumen by a single wider canal. These canaliculi are called *secretory capillaries*.

The microscopic appearance of the gland-cells changes with their periodic functional condition. In many glands all the cells exhibit simultaneously the same functional condition. In other glands, however, different functional conditions are encountered at the same time, even within the

FIG. 15.—PART OF A SECTION OF MUCOUS GLANDS OF THE TONGUE OF RABBIT. Blood-vessels injected. The nuclei of the gland-cells were only faintly visible in the preparation. $\times 180$. Techn. like No. 118 *b*.

FIG. 16.—SECTION OF FUNDUS GLAND OF MOUSE. Left upper half drawn after an alcohol preparation (Techn. No. 102), right upper half after a Golgi preparation (Techn. No. 119). The entire lower portion is a diagrammatic combination of both preparations.

same tubule or alveolus. The latter is the case in many mucous glands, the cells of which have delicate walls. In these, cells in a condition of activity and of exhaustion are found side by side in the same tubule. The loaded cells push the empty ones away from the gland-lumen; the latter then lie at the periphery of the tubule, and represent in this form the so-called "demilunes of Heidenhain" or "crescents of Giannuzzi" (Fig. 17). It must be remarked here that other authors regard the crescentic cells as young gland-cells destined to replace those that disintegrate in the secretory process. The absence of remnants of disintegrated cells contradicts this interpretation, as does also the impossibility of demonstrating karyokinetic figures. The nuclei of many glandular cells also exhibit a variable appearance according to the functional condition. In an empty cell the nucleus exhibits a delicate chromatin network and a conspicuous nucleolus (Fig. 17 *b*), while in loaded cells the nucleolus is invisible, and the chromatin network appears in the form of coarse fragments (Fig. 17 *a*).

The smaller branches of the ducts of many tubular glands must be regarded as belonging to the secretory follicles, since they are characterized by the specialized epithelium lining their walls, and participate in the function of secretion by eliminating certain materials (salts). They are thus not merely excretory ducts, but belong to the actively secreting portion of the glands. The difference in structure of these branches renders their division into two parts desirable. The first portion, proceeding from the terminal compartments, is narrow, and lined sometimes with flat, sometimes with cubical, cells. This is called the *intercalated or intermediate tubule*. The adjoining portion is wider

FIG. 17.—DIAGRAM OF THE ORIGIN OF THE CRESCENTS. Protoplasm shown darkly shaded, the secretion less shaded.

I. Cross-section of a tubule of a mucous gland, with 6 gland cells. 3 (a_1 , a_2 , a_3) are filled with secretion, and have pressed the three cells (b_1 , b_2 , b_3) empty of secretion, away from the gland lumen. Comp. Fig. 146.

II. Same section somewhat later. The cells, a_1 , a_2 , a_3 , have discharged a part of their secretion, and become still smaller. The cells, b_1 , b_2 , b_3 , again extend to the lumen and begin to secrete.

III. Same section still later. The cells, a_1 , a_2 , a_3 , have discharged the bulk of their secretion, and become still smaller. In the cells, b_1 , b_2 , b_3 , the secretion has accumulated to such an extent that they have become larger and compress their neighbors, a_1 , a_2 , a_3 .

IV. Same section still later. The cells, a_1 , a_2 , a_3 , are now entirely empty, and pushed entirely away from the gland lumen by b_1 , b_2 , b_3 , now full of secretion.

In I the cells b , in IV the cells a are the crescents.

and clothed with tall columnar cells, the bases of which show distinct longitudinal striation. These are called *intralobular tubes* or *secretory* (*salivary or mucous*) *tubes*. The relative length of the intercalated tubules and the intralobular tubes varies greatly in the different glands.

The *excretory ducts* consist of a simple or stratified columnar epithelium lining a wall of connective tissue mingled with elastic fibers.

The most complex glands consist of the following sections: (1) the excretory duct, which divides into (2) the secretory tubes, which lead into (3) the intercalated tubules, which pass into (4) the terminal compartments, which, finally, take up (5) the secretory capillaries.

II. THE CONNECTIVE TISSUES.

While in the epithelial tissues the cells constitute the principal mass, in the connective substances they are less noticeable, and instead the intercellular substance is conspicuously developed and variously differentiated. The predominance of the intercellular substance, which also functionally plays the more important part, is characteristic of the group of connective tissues. According to the nature of the intercellular substance they are divided into :

1. Connective tissue. 2. Cartilage. 3. Bone.

1. Connective Tissue.—The matrix or intercellular substance of connective tissue is more or less soft ; the cells are few in number. Several varieties are distinguished: (*a*) mucous connective tissue, (*b*) fibrillar connective tissue, and (*c*) reticular connective tissue.

(*a*) *Mucous connective tissue* consists of round or stellate branched cells and a great quantity of undifferentiated, muciferous intercellular substance con-

FIG. 18.—FROM A CROSS-SECTION OF THE UMBILICAL CORD OF A FOUR MONTHS' HUMAN EMBRYO. $\times 240$. 1. Cells. 2. Intercellular substance. 3. Connective-tissue bundles, cut obliquely, but at 4 directly cross-sectioned. Techn. No. 3.

FIG. 19.—CONNECTIVE-TISSUE BUNDLES OF VARIOUS THICKNESSES OF THE INTERMUSCULAR CONNECTIVE TISSUE OF MAN. $\times 240$. Techn. No. 4.

taining a few minute bundles of fine fibrils. In the higher animals it is found only in the umbilical cord of very young embryos, but is very common in many lower animals.

(*b*) *Fibrillar (areolar) connective tissue* consists of an abundant intercellular substance and cells.

The intercellular substance is differentiated into connective-tissue fibers, exquisitely fine (0.6μ) filaments united by a small quantity of homogeneous cement into bundles of varying thickness—connective-tissue bundles. These bundles are soft, flexible, slightly extensible, and characterized by their pale and indistinct contours, their longitudinal striation, their wavy course, and also by their chemical properties. On treatment with picric acid, they separate into their fibrils, swell up on the addition of dilute acids, *e. g.*, acetic acid, and become transparent. They are destroyed by alkaline fluids, and yield gluten on boiling.

The matrix of fibrillar connective tissue always contains *elastic fibers*, but in varying quantities (Fig. 20). In contrast to the connective-tissue bundles, they are characterized by their sharp dark outlines, their strong refractive

FIG. 20.—ELASTIC FIBERS. $\times 560$. *A*. Fine elastic fibers, *f*, from intermuscular connective tissue of man; *b*, connective-tissue bundles swelled by treatment with acetic acid. Techn. No. 10. *B*. Very thick elastic fibers, *f*, from ligamentum nuchae of ox; *b*, connective-tissue bundles. Techn. No. 11. *C*. From a cross-section of the ligamentum nuchae of ox; *f*, elastic fibers; *b*, connective-tissue bundles. Techn. No. 12.

power, and their conspicuous resistance to acids and alkalis. The elastic fibers vary from immeasurably fine to $11\ \mu$, and occur usually in the form of finer or coarser networks, the meshes of which are sometimes narrow, sometimes large (Fig. 21).

FIG. 21.—Network (*n*) of thick elastic fibers, on the left passing into a fenestrated membrane (*m*). From the endocardium of man. $\times 560$. Techn. No. 13.

FIG. 22.—*A*. Connective-tissue cells from intermuscular connective tissue $\times 560$. 1. Flat cell lying partly on a connective-tissue bundle; 2, folded cell; 3, cell of which the protoplasm is not visible; *b*, connective-tissue bundles. Techn. No. 5. *B*. Connective-tissue bundles with encircling fibers; *k*, nucleus. Techn. No. 8. *C*. Plasma-cells from the eye-lid of a child. Techn. No. 182.

Narrow-meshed networks composed of thick elastic fibers form the transition to elastic membranes, which are either homogeneous or finely striated and perforated with apertures of different sizes (hence the name fenestrated

membranes) and probably are produced by the merging of broad elastic fibers (Fig. 21).

When elastic fibers predominate over the connective-tissue bundles, the tissue is spoken of as *elastic tissue*. The elastic fibers are derived neither from cells nor from nuclei, but are a transformation of the matrix. In the beginning of their development they are thin, but thicken in the progress of their growth.

The connective-tissue cells are irregularly polygonal or stellate, flattened, and variously bent or folded (Fig. 22, *A*). The flattening and bending are explained by the adaptation of the cells to the narrow spaces between the connective-tissue bundles. Flattened cells not infrequently form sheaths about the connective-tissue bundles. If such a bundle be treated with acetic acid it swells and bursts the ensheathing cells, of which encircling pieces are often retained and constrict the swelled bundle. Formerly these remnants of cells were considered fibers, and were called "encircling fibers" (Fig. 22, *B*). Other connective-tissue cells are rounded, rich in protoplasm, coarsely granular, and comparatively of large size. These are termed *plasma-cells*, and are found especially in the neighborhood of small blood-vessels (Fig. 22, *c*). Others again, the "Mastzellen," are characterized by the affinity of their protoplasm for certain anilin dyes (*e. g.*, dahlia) but do not stand, as their name might suggest, in any demonstrable relation to the processes of nutrition. [They are also known as *granule-cells*.] The protoplasmic body of the connective-tissue cells encloses a nucleus and often contains pigment-granules; in the latter case they are called *pigment-cells*. These are found in man only in certain parts of the skin and in the eye, but in the lower animals they are very common. Con-

FIG. 23.—FAT-CELLS FROM THE AXILLA, *A*, OF A LEAN INDIVIDUAL. $\times 240$. 1. In focusing the equator of the cell; 2, objective somewhat elevated; 3, 4, forms changed by pressure; *p*, traces of protoplasm in the vicinity of the flat nucleus, *k*. *B*, Of an emaciated individual; *k*, nucleus; *f*, fat-drops; *c*, blood-capillaries. *C*, connective-tissue bundles. Techn. No. 9.

nective-tissue cells may contain fat-globules which, if very large, coalesce and give a spherical form to the cell, which is then designated a *fat-cell* (Fig. 23, *A*). In such cells the protoplasm occupies only a narrow peripheral zone, in which lies the extremely flattened nucleus. This protoplasmic zone is often so thin as to be invisible. Aggregations of fat-cells are abundantly supplied with blood- and lymph-vessels and nerves, and form what is called *adipose tissue*, which bears a very important relation to metabolism. In cases of extreme emaciation the fat in fat-cells is reduced to a few small globules. In place of the fat which has disappeared there is a pale protoplasm mixed with a mucoid fluid. The cell is no longer spherical, but has become flattened, and

is known as a *serous fat-cell* (Fig. 23, *B*). In many fat-cells after death spherical masses of needle-shaped crystals appear—the so-called *margarin crystals*.

In addition, smaller irregularly-spherical cells are found in connective tissue that are not connective-tissue elements, but leucocytes that have passed out of the blood-vessels. They are described as “wandering cells,” in distinction to those of the connective tissue, which are designated as “fixed;” but this classification cannot be rigidly carried out, since in certain conditions (mainly pathologic) the fixed connective-tissue cells, and also epithelial and glandular cells, can migrate, and it is therefore better to term the latter “histogenetic,” the leucocytes “hematogenetic” wandering cells.

The number and distribution of the different kinds of cells is subject to considerable fluctuation.

The different elements of fibrous connective tissue are united either without any definite arrangement, as in areolar tissue, or are regularly disposed in definite structures. Areolar tissue is distinguished by its loosely-connected bundles of fibers interlacing in every direction; it occurs between neighboring organs, and serves to connect them and fill in the interspaces. For this reason it is also called “interstitial” tissue. The cells of areolar tissue not infrequently contain fat. The fibrous connective tissues characterized by closer connection and regular arrangement of the bundles comprise: the corium, the serous membranes, the periosteum, the perichondrium, the tendons, fasciæ, and ligaments; the compact sheaths of the central nervous system, of the blood-vessels, of the eye, and of many glands.

The fibrous connective tissue in immediate contact with epithelium is usually modified, forming a structureless membrane called *basement-membrane* or *membrana propria*, also *hyaloid membrane*. The membrana propria of many glands—for example, salivary glands—consists of basket-works of flattened, often stellate, cells, which surround the gland-tubules.

(c) *Reticular Connective Tissue*.—The views in regard to the structure of reticular connective tissue are divided. According to an opinion formerly widely entertained, it consists of a delicate network of anastomosing stellate cells, and to this may be traced the name “cytogenous,” that is, formed of cells. Accordingly, mucous tissue may be termed cytogenous tissue. There is no doubt but that such networks occur in lower animals and in embryonic stages of higher animals. In the higher vertebrates, however, the relations are changed; in these the network consists of slender bundles of fibrillar connective tissue, upon which lie flattened, nucleated cells (Fig. 24). By means of com-

FIG. 24.—RETICULAR CONNECTIVE TISSUE. From a shaken section of a human lymph-gland. $\times 560$. Techn. No. 48.

plicated methods the outlines of the cells on the fibers can be demonstrated. Finally, the fact that fibrillar connective tissue, even in the adult, may change into reticular tissue can only be comprehended on the assumption that the latter is a network of delicate fiber-bundles. The meshes of reticular connective tissue are usually crowded with leucocytes. It occurs principally in lymph-glands (better, lymph-nodules), and is then called *adenoid* tissue.

2. Cartilage.—The matrix of cartilage is dense, elastic, easily cut, and milk-white or yellowish in color. The cells present little that is characteristic in form; they are usually spherical or, from being flattened on one side, somewhat angular. They lie in the spaces or *lacunæ* of the matrix, which they fill completely. Whether, as in bone, the matrix is penetrated by a system of minute channels communicating with and connecting the lacunæ is extremely

FIG. 25.—HYALINE CARTILAGE. $\times 240$. *A*. Surface view of the ensiform process of frog, fresh; *l*, protoplasm of cartilage-cell, which entirely fills the lacuna; *k*, nucleus; *g*, hyaline matrix. Techn. No. 14. *B*. Portion of cross-section of human rib-cartilage several days after death; examined in water: the protoplasm, *l*, of the cartilage-cells has withdrawn from the walls of the lacunæ, *k*; the nuclei are invisible. 1. Two cells within one capsule, *k*; *x*, a developing partition. 2. Five cartilage-cells within one capsule; the lowest cell has fallen out, and here only the empty space is seen. 3. Capsule cut obliquely, and apparently thicker on one side. 4. Capsule not cut, but showing the cell within. *g*. Hyaline matrix transformed into rigid fibers, *f*. Techn. No. 15.

doubtful. Many observations, in which such channels were apparently seen, have been acknowledged as erroneous; the supposed channels were a result of shrinkage, and can be produced by treating cartilage with absolute alcohol or ether. Not infrequently the matrix immediately surrounding the lacunæ is specialized, and forms a strongly refractive, occasionally concentrically-striated *capsule*. The otherwise homogeneous matrix may be free from admixture of fibrous tissue, or it may be penetrated by elastic fibers or by bundles of white fibers. Accordingly, three varieties are distinguished: (*a*) *hyaline cartilage*, (*b*) *elastic cartilage*, (*c*) *fibro-cartilage*.

(*a*) *Hyaline cartilage* is of a faint bluish, pearly, transparent color. It occurs as the cartilages of the respiratory organs and of the nose, as the costal and the articular cartilages, also in the synchondroses, and in the embryo in

many situations where it is later replaced by bone. It is characterized by the homogeneity of its matrix, which in the ordinary methods of investigation appears amorphous throughout, but after special processes, *e. g.*, artificial digestion, falls apart into bundles of fibers. Further evidence in confirmation of its fibrillar structure is afforded by its appearance when examined in polarized light. It is very firm, very elastic, and on boiling yields chondrin.

In certain cases the matrix may undergo a peculiar modification. In the thyroid and costal cartilages it is transformed patchwise into rigid fibers, which impart an asbestos-like lustre, perceptible on macroscopic inspection. In advanced age deposition of calcareous salts may take place in the hyaline matrix, in the beginning appearing in the form of minute granules, subsequently as complete husks, surrounding and enclosing the cells. In the cartilages of the larynx this may occur as early as the twentieth year.

The cells of hyaline cartilage frequently occur in groups or nests, an arrangement explained by the conditions and processes of growth. Two cells may lie in one lacuna and be enclosed within the same capsule (Fig. 25, *B 1*);

FIG. 26.—ELASTIC CARTILAGE. $\times 240$. 1. Portion of section of vocal process (anterior angle) of arytenoid cartilage of a woman thirty years old; the elastic substance in the form of granules. 2 and 3. Portions of sections of epiglottis of a woman sixty years old; a fine network of elastic fibers in 2, a coarser network in 3. *z*, Cartilage-cell, nucleus not visible; *k*, capsule. Techn. No. 16.

they are the descendants of the original cell, which has undergone division by the indirect mode; in other cases, a thin partition of hyaline substance may be seen between them. In still other cases, the septum does not develop immediately, and the process of cell-division may be repeated, until groups of four, eight, and even more cells may be enclosed within one capsule (Fig. 25, *B 2*). Such phenomena were supposed to establish a special theory of cell-division, the so-called endogenous cell-formation. Not infrequently the cartilage-cells in adults contain oil-globules.

(*b*) *Elastic cartilage* has a faint yellowish color. It occurs as the cartilages of the external ear, of the epiglottis, of Wrisberg and Santorini, and of the vocal process (anterior angle) of the arytenoid cartilages. It presents the same structural peculiarities as hyaline cartilage, but is distinguished by the networks of finer or coarser elastic fibers that penetrate the matrix. The elastic fibers do not arise directly from the cartilage-cells, but by a transformation of the matrix, and appear in the vicinity of the former as minute granules.

which later are disposed in linear rows and fuse into fibers. This phenomenon, according to an opposite view, is regarded as an indication of post-mortem disintegration of the elastic fibers.

(c) *Fibro-cartilage* is found in the intervertebral disks, the pubic symphysis, the inferior maxillary and sterno-clavicular articulations. The matrix contains an abundance of fibrous connective tissue in loose bundles extending in all directions (Fig. 27, *g*). The cartilage-cells are few in number, have thick capsules, and occur in small groups or rows at comparatively wide intervals.

FIG. 27.—FROM A HORIZONTAL SECTION OF THE INTERVERTEBRAL DISK OF MAN. *g*, Fibrillar connective tissue; *z*, cartilage-cell (nucleus invisible); *k*, capsule surrounded by calcareous granules. $\times 240$. Techn. No. 17.

3. **Bone.**—The matrix of bone, osseous tissue, is distinguished by its hardness, solidity, and elasticity, properties due to an intimate blending of organic and inorganic substances. This union is of such a nature that either part may be removed without destroying the tissue. On treatment with acids, the inorganic substances are withdrawn; the bone is decalcified, is rendered flexible, and is easily cut, like cartilage. On the other hand, the organic substances may be removed by cautious heating; the bone is then said to be calcined. Fossil

bones, similarly, are deprived of the organic substances through the prolonged action of moisture. The matrix or ground-substance is composed of calcium salts, especially basic calcium phosphate, and of collagenous fibrils, united by a

FIG. 28.—FROM A GROUND SECTION OF DRIED BONE OF ADULT MAN. *h*, Lacunæ; *k*, canaliculi; *g*, bone-matrix. *A*, Seen from the surface. *B*, Seen from the side. $\times 560$. Techn. No. 55.

FIG. 29.—FROM SECTIONS, *a*, OF THE HUMERUS OF A FOUR MONTHS' HUMAN EMBRYO; *b*, of the middle turbin bone of man; *z*, bone-cells lying in the lacunæ, *h*; the canaliculi are only slightly visible; *g*, matrix. $\times 560$. Techn. No. 61.

small amount of cement-substance in finer or coarser bundles; accordingly, a *compact*, or lamellar, and a *spongy* matrix are distinguished. It appears homogeneous or faintly striated and contains numerous spindle-shaped spaces 15 to 27 μ in length—the *lacunæ*—from which minute branched channels—the *canaliculi*—radiate in all directions; the lacunæ, with their minute canals, form an intercommunicating system of lymph-spaces throughout the matrix. Within the lacunæ, sometimes improperly called “bone-cells,” lie nucleated

flattened bodies, the real *bone-cells*. It is extremely doubtful whether in the adult bone the cells are connected by means of processes extending through the canaliculi, although such connection is readily observed in developing bone.

The skeleton of the adult is formed principally of compact bone, which is characterized by the arrangement of the fiber-bundles in lamellæ; the matrix contains elastic fibers. Spongy bone occurs in the fetus as periosteal and intermembranous bone, and is found in the adult along sutures and at the points of insertion of tendons; it always contains uncalcified connective-tissue bundles, the so-called Sharpey's fibers, which, however, are also found in the circumferential and interstitial lamellæ of compact bone, the remains of the primary or periosteal bone.

Fibrous connective tissue and cartilage may be converted directly into osseous tissue by calcification of the matrix; and the connective tissue and

FIG. 30.—PORTION OF CROSS-SECTION OF THE DIAPHYSIS OF THE HUMERUS OF A FOUR MONTHS' HUMAN EMBRYO. $\times 560$. Techn. No. 61.

cartilage-cells then become bone-cells. This, however, is of comparatively rare occurrence; usually, the formation of osseous tissue follows the calcification of the matrix of the embryonal cartilage and connective tissue, in which young, still indifferent, connective-tissue cells arrange themselves upon the surface of the calcified trabeculæ and produce bone-substance.

Dentine is a modification of bone, from which it is distinguished by its developmental history; the formative cells, the *odontoblasts*, are not enclosed within the matrix, but penetrate the latter with their processes. Further details will be found in connection with the structure of teeth.

Blood-vessels, Lymphatics, and Nerves.—Connective-tissue structures are, in general, poorly supplied with blood-vessels, lymph-vessels, and nerves. An exception occurs in adipose tissue, which has a rich vascular supply. Connective tissue plays a very important part, however, in the transference of nutritive

fluids—*tissue juices*, *lymph*—passing from the blood-vessels to the tissues. When the matrix is soft, as in mucous tissue, the lymph permeates the entire substance; when on the other hand, it is denser, the lymph circulates in a system of intercommunicating channels formed by the cell-spaces—*lymph-spaces*—and the minute canals connecting them—*lymph-canalliculi*. This is the case in bone and the more compact connective tissues. Whether the tissue-juice is diffused throughout the matrix of hyaline cartilage or conveyed in definite channels is still undetermined. The intercellular substance of epithelium is in direct connection with the lymph-capillaries of the subjacent connective-tissue, and may be regarded as being similarly permeated by the lymph.

III. THE MUSCULAR TISSUES.

The structural elements of the muscular tissues, the *muscle-fibers*, occur in two forms, the *smooth* and the *striated*. Both are cells whose body is extraordinarily elongated.

1. *Smooth, Non-striated, or Involuntary Muscle*.—This consists of contractile fiber-cells, spindle-shaped, cylindrical, or slightly-flattened elements with tapering extremities (Fig. 31). They vary in length from 45 to 225 μ . in

FIG. 31.—TWO SMOOTH MUSCLE-FIBERS FROM SMALL INTESTINE OF FROG. $\times 240$. Isolated with 35 per cent. potash-lye. The nuclei have lost their characteristic form through the action of the lye. Techn. No. 24.

width from 4 to 7 μ ; in the gravid uterus fibers measuring 0.5 mm. have been found. They are composed of homogeneous protoplasm and an elongated or rod-shaped nucleus; the latter is characteristic of the smooth muscle-fiber. The protoplasm of certain fibers, those, for example, of the vas deferens, exhibits longitudinal striation, which has led some authors to regard the smooth muscle-fiber as composed of minute contractile fibrillæ. In fishes and amphibians muscle-fibers containing pigment have been found in the iris. [According to many histologists the smooth muscle-fiber is invested by an exceedingly delicate structureless hyaline sheath, corresponding to the sarcolemma of the striated fiber.]

The fibers are collected into fasciculi, and firmly held together by a homogeneous cement-substance. Communication between neighboring fibers by means of protoplasmic processes or intercellular bridges, like those occurring in certain epithelia, has been observed in the muscular tunic of the intestine of the dog and cat, and also of man. Septa of connective tissue are found only at comparatively wide intervals (Fig. 32).

The fasciculi are united to form strata or membranes, in which their disposition is parallel, as in the muscular coat of the intestine, or they cross and interlace forming complicated networks, as in the urinary bladder and the uterus. The larger blood-vessels run in the connective-tissue septa; but the

capillaries penetrate the fasciculi, within which they form networks with elongated meshes. The lymph-vessels follow the course of the blood-vessels, and are present in considerable numbers.

For the nerves of smooth muscle, see *Peripheral Nerve-endings*.

Smooth muscle-tissue occurs in the alimentary canal, in the trachea and bronchial tubes, in the gall-bladder, in the capsule and pelvis of the kidneys, in the ureters and the urinary bladder, in the reproductive organs, in the vascular channels and lymph-vessels, in the eye, and in the skin. The contraction of smooth muscle-fiber is slow, and is not under the control of the will.

2. *Striated or Voluntary Muscle*.—It is only by the study of their development that the striated muscle-fibers can be recognized as the morphologic equivalents of cells. As a result of the extraordinary elongation of the embryonal elements, the proliferation of their nuclei, and the peculiar differentiation of their protoplasm they have become highly-specialized structures. The fibers are cylindrical in shape, and in the interior of the larger muscles have rounded or pointed ends; on the other hand, at the extremities of the muscle they possess a pointed inner end, while the outer end, in contact with the tendon, is broad. The latter is blunt or notched, often step-like and tapering. Anastomoses, divisions, and fissures occur; branched fibers are found in the muscles of the eye, the tongue, and the skin (Fig. 34, 4). They vary in length from 5.3 to 12.3 cm., in width from 10 to 100 μ . It is probable that there are fibers having greater length, but their isolation entire is very difficult to accomplish. In the embryo the fibers differ but little in width, but after birth their development in this dimension varies, and is dependent on the functional activity of the muscle; in the adult, robust muscles possess thick fibers, delicate muscles have thin fibers. Apart from this, their diameter depends also on the nutritional condition of the individual. Furthermore, larger animals possess thicker fibers than smaller ones. The difference in caliber is, therefore, of a threefold nature.

Under the microscope each fiber exhibits alternate broad *dim* and narrower *light* transverse striæ. The substance of the dim stripes is doubly refracting or *anisotropic*, that of the light stripes singly refracting or *isotropic*. High amplification shows that both the dim and light striæ are transversely divided; in the light zone a delicate dim interrupted line may be seen, the *intermediate disk* (Fig. 33, 9). Each part of the light zone, above or below the intermediate disk, constitutes a *lateral disk*. In the dim transverse band a clear stripe, the *median disk*, has been observed. [In certain forms of invertebrate muscles the lateral disk is crossed, above and below the intermediate disk, by a dark stripe, the *secondary disk*.] Owing to their extreme variation and their instability, these disks are of subordinate significance. Besides the cross-marking, a more

FIG. 32.—SECTION OF THE CIRCULAR LAYER OF THE MUSCULAR COAT OF THE HUMAN INTESTINE. $\times 560$. Techn. No. 103.

or less distinct longitudinal striation may be observed. Treatment with chromic acid solutions renders this striation more evident, and may even effect the disintegration of the fibers, which fall apart lengthwise into delicate fibrils, each of which exhibits the cross-striæ. These fibrils are the contractile structural elements, and are called *ultimate fibrillæ*.

The muscle-fibers of some animals, after treatment with certain reagents, cleave transversely into disks. Both fibrillæ and disks may be further separated into smaller prismatic anisotropic particles called *sarcous elements*. Certain authors have interpreted the disks, others the sarcous elements, as the true structural units.

[According to the theory of Rollett regarding the structure of voluntary muscle, the fiber is composed of the dim anisotropic *contractile substance* and the light isotropic, relatively passive *sarcoplasm*. The highly-specialized contractile substance is in the form of slender spindles, the ends of which are prolonged into extremely fine filaments and terminate in a minute knob. The

FIG. 33.—*B*, PORTION OF MUSCLE-FIBER OF MAN: *a*, anisotropic; *i*, isotropic band; *q*, intermediate disk; *k*, nucleus. $\times 560$. Techn. No. 18. *A*, Muscle-fiber of frog; *f*, fibrillæ; *k*, nucleus. $\times 240$. Techn. No. 21.

spindles, arranged end to end, form the continuous *contractile fibrillæ*, which grouped in parallel bundles constitute the sarcostyles, and extend throughout the length of the fiber. The thicker parts of the apposed spindles form the dim transverse bands, the knobs the dim intermediate disks in the light transverse bands. The spindles correspond to the sarcous elements.]

The contractile fibrillæ are grouped into bundles—*sarcostyles* or *muscle-columns*; they are arranged parallel to one another and held together by the sarcoplasm, which also surrounds and unites the bundles. The disposition of the sarcoplasm is best seen in cross-section; high amplification is required. It presents the appearance of a clear network, and within the meshes are the muscle-columns in section,—small dark polygonal areas known as *Cohnheim's fields*. The sarcoplasm contains the *interstitial granules*—consisting partly of fat and probably also partly of lecithin—and the *nuclei*. The latter are oval bodies placed parallel to the long axis of the fiber; in mammals, bony fishes, and some birds they are chiefly situated immediately beneath the sarcolemma,

upon the surface of the muscle-substance; in other vertebrates they are embedded within the sarcoplasm.

Each muscle-fiber is closely invested by a structureless sheath, the *sarcolemma*, which represents the cell-membrane. Thus the fiber of striated muscle comprises the fibrillæ, the sarcoplasm, the muscle-nuclei, and the sarcolemma.

The striated fibers are found in the muscles of the trunk and the extremities, of the eye and the ear, also in the tongue, the pharynx, the upper half of the œsophagus, in the larynx and the diaphragm, the genital organs, and the rectum.

In some animals, the rabbit, for example, two varieties of striated muscles are distinguished, the *red* (semitendinosus, soleus) and the *white* or pale (adductor magnus); and correspondingly, two varieties of muscle-fibers: 1.

FIG. 34.—PORTIONS OF ISOLATED STRIATED MUSCLE-FIBERS OF FROG. $\times 50$. 1. After treatment with water: *s*, sarcolemma; at *x* the muscle-substance is torn: the cross-striation not apparent, the longitudinal striation distinct. Techn. No. 19. 2. After treatment with acetic acid: *k*, nuclei; the minute punctations represent interstitial granules. Techn. No. 20. 3. After the action of concentrated potash solution: *c*, rounded ends; the numerous nuclei are swollen and vesicular in appearance. With this amplification the cross-striation in 2 and 3 is not visible. Techn. No. 22. 4. Branched muscle-fiber from the tongue of the frog.

dim fibers, rich in sarcoplasm, less regularly cross-striated, exhibiting distinct longitudinal striation, and possessing in general a smaller diameter (found in the soleus of the rabbit); 2, pale fibers, poor in protoplasm, more distinctly cross-striated, and having in general a greater diameter. The latter represent the more highly-differentiated fibers. While in certain animals the two varieties of fibers occur separately, each in particular muscles, in others—also in man—they are found intermingled in the same muscle. As a rule, the more functionally active muscles—cardiac, ocular, masticatory, and respiratory—contain the greater number of red fibers. The pale fibers, on the other hand, respond more rapidly to electric stimulation.

The contraction of the striated fibers, as compared with that of smooth muscle, is quick, and is under the control of the will. The striated fibers are united into bundles by areolar tissue, which serves also to convey the numer-

ous ramifications of the blood-vessels and nerves supplying the muscular tissue. The lymphatic vessels are few in number.

3. *Cardiac Muscle*.—The muscle-fibers of the heart occupy a peculiar position. Although transversely striated, in the history of their development, as well as histologically, they must be regarded as modifications of the smooth muscle-fibers. In the lower vertebrates, in frogs for example, they are spindle-shaped, possess elongated nuclei, and are often more distinctly striated transversely than longitudinally (Fig. 35, *A*).

The cardiac muscle of mammals consists of short, cylindrical fibers, the ends of which are often step-like. The protoplasm is partially differentiated into cross-striated *fibrille*, which not infrequently are grouped into *muscle-columns* radially arranged to the axis of the fiber (Fig. 35, *D*). The remnant of undifferentiated protoplasm—*sarcoplasm*—proportionately consid-

FIG. 35.—*A* and *B*, MUSCLE-FIBERS OF HEART, isolated in potash-lye. *A*. Of frog. *B*. Of rabbit; x, lateral branches. $\times 240$. Techn. like No. 22. *C*. In longitudinal section. *D*. From a cross-section of papillary muscle of man. *C* magnified 240, *D*, 560 diameters. Techn. No. 33.

erable in comparison with that of striated voluntary fibers, is found chiefly in the axial part of the fiber, from which processes radiate between the muscle-columns. Longitudinal striation is often marked, owing to the generous amount and the disposition of the sarcoplasm. The oval nucleus is embedded in the axial part of the sarcoplasm, which frequently contains pigment-granules or oil-droplets. A cell-membrane or sarcolemma is wanting. The cardiac muscle of the higher animals is characterized by the anastomosis of the cells by means of short, lateral processes. [The cells are joined end to end, transverse lines of cement-substance indicating the line of union between the individual elements.]

IV. THE NERVOUS TISSUES.

The elements of the nervous tissues in an early embryonic stage are, without exception, cells having a spherical form; they are called *neuroblasts*. In the course of their development they become elongated and pyriform, and the narrow end grows out as a long, delicate process (nerve-process), often

extending to the length of a meter; it terminates in a free branched end, and is named *axis-cylinder process*. From the body of the cell—now termed a *nerve- or ganglion-cell*—other processes may arise, which, however, are short and divide dichotomously; these are called *protoplasmic or ramifying processes* (dendrites). The axis-cylinder process also may have delicate lateral branches, the *collateral fibrils*. The nerve-cell and axis-cylinder process together constitute the *neuron*; the dendrites and collateral fibrils may be regarded as secondary processes of the neuron, forming with the latter the *neurodendron*.

The axis-cylinder process may be naked throughout its extent, or it may be invested by different sheaths; these are the *neurilemma*, or sheath of Schwann, and the *medullary sheath*, or white substance of Schwann. Both, originally alien to the nervous tissues, are derived from connective tissue; both invest the axis-cylinder only in a portion of its course. There are stretches in which the axis-cylinder is entirely without investment, is naked (Fig. 36, *a*); stretches in which it is enveloped by either the neurilemma (Fig. 36, *b*) or the medullary substance (Fig. 36, *c*), and, finally, stretches in which both sheaths are present (Fig. 36, *d*); in the latter case the medullary sheath is always the innermost envelope, lying directly upon the axis-cylinder, and is itself ensheathed by the neurilemma. The axis-cylinder always occupies the longitudinal axis, hence its name. Owing to the often great length of the axis-cylinder, it is not possible to investigate the neuron as a whole.

As a rule, it is seen only in fragments, either the nerve-cell or the axis-cylinder, and this explains the former division of the elements of the nervous tissues into

FIG. 36.—DIAGRAM OF A NEURON.

nerve-cells and *nerve-fibers*—the latter being the axis-cylinder processes with their sheaths. There are no independent nerve-fibers, each so-called fiber is a process of a ganglion-cell. However, for practical reasons, the old classification is retained.

NERVE-CELLS.

Nerve, or ganglion-cells, are found in the ganglia, in the organs of special sense, along the course of cerebro-spinal, as well as sympathetic nerves, but principally in the central nervous system. They differ greatly in size (4 to 135 μ and more) and in form. There are spherical and spindle-shaped ganglion-cells, and irregularly-stellate forms are very common; the latter are those in which the protoplasm sends off numbers of processes and so gives rise to the stellate outlines. Ganglion-cells having two processes are termed *bipolar*,

FIG. 37.—VARIOUS FORMS OF GANGLION-CELLS. $\times 240$. 1. Bipolar cell from the ganglion acusticum of an embryo rat. Techn. No. 187. 2. Multipolar cell from the spinal cord of man. Techn. No. 26. 3. Cell from the Gasserian ganglion of man, axis-cylinder process torn off. Techn. No. 25. 4. Cell with T-branched from a spinal ganglion of a young rat. Techn. No. 70.

those having several processes, *multipolar* ganglion-cells (Fig. 37). There are also *unipolar* ganglion cells; these occur in the sympathetic nerve of amphibians and universally in the olfactory mucous membrane. They possess, in fact, but a single process. The nerve-cells of the spinal ganglia, on the other hand, are only apparently unipolar; bipolar in the embryo, in the course of development they become unipolar by the gradual approach of the processes, which eventually come off from the cell by a common stalk, from which they then diverge at right or obtuse angles. These are the cells described as having T-shaped or Y-shaped processes. Apolar cells, that is, ganglion-cells without processes, are

either immature forms or artificial products, the processes in the latter case having been torn off in the manipulation required for isolation.

The ganglion-cells are composed of granular or finely-striated protoplasm and have a characteristic vesicular nucleus, poor in chromatin and enclosing a conspicuous nucleolus. The protoplasm not infrequently contains yellowish-brown pigment-granules (Fig. 37). A cell-membrane is wanting.

The processes of nerve-cells are of two kinds: 1, the *axis-cylinder* (Deiters's) and, 2, the branched *protoplasmic* processes (Fig. 38). They are most readily distinguished in the multipolar cells. The axis-cylinder—usually the only process of the kind—is the first outgrowth from the embryonal spherical cell, and is characterized by its hyaline appearance and smooth outlines; its course is cellulifugal—it leads from the cell. The protoplasmic processes—usually several in number—are a later outgrowth of the embryonal cell, are thicker, granular or finely striated, and often varicose; their course is cellu-

lipetal—toward the cell. They undergo repeated dichotomous division and terminate in an intricate network of extremely fine fibrils. Cells possessing more than one axis-cylinder process occur in the brain of the rabbit, in the substantia gelatinosa of the spinal cord of the chick, and perhaps, also, in the sympathetic nerves of the higher vertebrates. In bipolar cells (spinal ganglion-cells of the lower vertebrates and embryos) in which both processes become the axis-cylinders of medullated nerve-fibers, the fiber going toward the central nervous system corresponds to the axis-cylinder process, the peripheral fiber to a protoplasmic process.

FIG. 38.—NERVE-CELL (CELL OF PURKINJE) FROM A SECTION THROUGH THE HUMAN CEREBELLAR CORTEX.
 X 180. Techn. No. 74.

Dependent on the behavior of the axis-cylinder process, two kinds of ganglion-cells are distinguished—*cells of the first type*, having a long axis-cylinder process which becomes the axis-cylinder of a medullated nerve-fiber, and *cells of the second type*, having a short axis-cylinder process which divides and subdivides and terminates in a nervous ramification in the vicinity of the cell (Fig. 39). The axis-cylinder process of cells of the first type, after giving off a number of fine branched twigs, the collateral fibrils, and running an extended course, often embracing many centimeters, as the axis-cylinder of a

nerve-fiber, undergoes rapid division and terminates in a plexus of delicate fibrils. All the processes terminate in free endings, without forming anastomoses; there is no connection between the processes of adjacent cells except by contact. There is, therefore, properly no nervous network, but only a dense felt-work of interlacing fibrils. There may be some exceptions; in recent investigations of the retina and of the electric organ of the torpedo nervous networks formed by the processes of several nerve-cells have been described. In general, the phrase "nervous network" or "nervous plexus" is to be interpreted as signifying the disposition of single nerve-fibers that branch off from nerve-fiber bundles to join other bundles. The transition of one nerve-fiber into another never occurs.

FIG. 39.—TWO NERVE-CELLS FROM THE SPINAL CORD OF AN EMBRYO CHICK SEVEN DAYS OLD. The axis-cylinder or nerve-process of the left cell is not seen in its entire length. $\times 200$. Techn. No. 70.

NERVE-FIBERS.

Dependent upon the presence or absence of the medullary sheath, nerve-fibers are divided into the *medullated* or white and the *nonmedullated* or gray. Each division is susceptible of a subdivision dependent on the presence or absence of the neurilemma.

Nonmedullated Fibers. *Without a Neurilemma.*—These consist of the naked axis-cylinder only, and are found in the olfactory nerves, where they are held together and grouped into bundles by connective tissue. Similar are many fibers of the sympathetic nerve, the so-called Remak's fibers; they

are transparent, cylindrical, or band-like in form, from 3 to 7 μ wide, about 2 μ thick, and exhibit faint longitudinal striation; they are similarly grouped into bundles, which possess an imperfect sheath formed by closely applied flattened connective-tissue cells having oblong nuclei.

While the fibers described above exhibit the same structure throughout their length, there are, on the other hand, nerve-fibers of which only certain divisions are naked axis-cylinders; such divisions occur as the peripheral endings of the nerves of special sense, in sensory as well as motor nerves, and as the proximal portion, coming off from the cell as the axis-cylinder process (Fig. 36, *a*).

Nonmedullated Nerve-fibers. *With a Neurilemma.*—These consist of the axis-cylinder enveloped by a neurilemma, and are of the same structure throughout their extent; they are found in many invertebrates, and among vertebrates, in amphioxus, and in cyclostoma. They occur in limited portions in the course of the cerebro-spinal nerve-fibers (Fig. 36, *b*).

Medullated Nerve fibers.—Among these is none which possesses the medullary sheath throughout its length; this always invests only one portion of the fiber. The medullated fibers may be *without a neurilemma*, and consist of the axis-cylinder and the medullary sheath; such fibers occur only in the central nervous system. Medullated fibers *with a neurilemma* are found in the trunks and branches of the cerebro-spinal nerves, also in the sympathetic nerve, and vary in thickness from 1 to 20 μ . The thickness of the nerve-fiber bears no relation to its motor or sensory nature, but appears to be determined by its length: the longer its course, the thicker is the fiber. Division of the medullated fibers occurs (1) throughout the central nervous system, principally where the collateral fibrils diverge at right angles into the white substance; and (2) in the peripheral nervous system shortly before their ultimate distribution (Fig. 36).

The medullated nerve-fibers have a brief lease of life. They degenerate by a gradual breaking down of the white substance and the axis-cylinder into a granular mass containing numerous nuclei; in this mass both parts are regenerated, the axis-cylinder probably by outgrowth of the axis-cylinder process of the nerve-cell.

The **axis-cylinder**, the essential part of every nerve-fiber, occasionally exhibits a delicate longitudinal striation, the indication of its fibrillar structure. Each fibrilla represents a special conducting path and is cemented to neighbor-

FIG. 40.—TEASED PREPARATION FROM THE SYMPATHETIC NERVE OF RABBIT. 1, Non-medullated; 2, thin medullated nerve-fibers; 3, ganglion-cell; characteristic appearance of the nucleus lost after treatment with osmic acid; 4, nuclei of connective-tissue capsule; 5, fine connective-tissue fibers. $\times 240$. Techn. No. 32.

ing fibrillæ by a small amount of finely-granular interstitial substance—*neuropiasm*. [A delicate, elastic, special investment of the axis-cylinder—the axilemma—is described by Kühne. By some authors it is regarded as an artifact.]

The *medullary sheath* is composed of a semi-fluid, highly refracting, fatty substance, the *myelin*, which imparts to fresh medullated fibers the appearance of glistening hyaline cylinders, homogeneous throughout, the structure of which can only be perceived by the help of reagents.

In favorable conditions it may be seen that the medullary sheath is not continuous, but is divided at slightly irregular intervals by oblique incisions or clefts into small conical or funnel-shaped pieces, the *Schmidt-Lantermann segments* (medullary segments, cylindro-conical segments), which are united by cement-substance (Fig. 41, 9). Kölliker has latterly interpreted these oblique

FIG. 41.—MEDULLATED NERVE-FIBERS FROM THE SCIATIC NERVE OF FROG. $\times 280$. 1. Normal; 2, shrunken; 3, tortuous axis-cylinder; 4, node of Ranvier; 5, neurilemma with nucleus. Techn. No. 29. 6, 7, 8, and 9, fresh medullated nerve-fibers; 10, post-mortem distortion of medullary substance; *r*, annular constriction; *i*, incisures of Lantermann; *i*, medullary segment. Techn. No. 27 a.

markings as artifacts. After treatment with different reagents, the apparently homogeneous medullary substance of fresh nerve-fibers in dying undergoes partial transformation, and the fibers exhibit a characteristic double contour (thence the old designation, “double-bordered,” or “dark-edged” fibers), and later appear mottled, owing to the distortion of the white substance, which collects into irregular spherical masses (Fig. 41, 10). [According to Kühne and Ewald the medullary substance consists of two parts: a reticulum composed of a resistant material resembling neurokeratin, which encloses within its meshes the other part—the myelin. Owing to the variability in the appearance of the network, other authorities regard it as an effect of the reagents employed to demonstrate it.]

At regular intervals along the medullated nerve-fibers the medullary sub-

stance is interrupted, so that the axis-cylinder and neurilemma come into contact. At these points the fibers exhibit well-marked annular constrictions, termed the *nodes of Ranvier* (Fig. 42). These constrictions occur in all peripheral medullated fibers, at intervals of from 0.08 mm. in thin, to 1 mm. in thick fibers, dividing them into *internodal segments* or *internodes*. In the vicinity of the nodes the axis-cylinder frequently shows a biconical enlargement, probably due to a local accumulation of neuroplasm. After treatment with silver nitrate, the nodes are rendered conspicuous by a dark annular disk called the constricting band, produced by the staining of the cement-substance collected at these points, and distinct transverse striæ (Frommann's lines) appear on the adjacent parts of the axis-cylinder. [The stained cement-substance forms the transverse bar, the stained axis-cylinder the vertical bar of a minute dark-brown cross ("silver cross"). The division of a medullated nerve-fiber always occurs at the site of a node of Ranvier.]

The *neurilemma*, or sheath of Schwann, is a delicate structureless membrane, against the inner surface of which lie oval nuclei surrounded by a very small amount of protoplasm (Fig. 41, 5).

The union of the elements of the nervous tissues in the peripheral nervous system is secured by means of connective tissue, which contains the ramifications of the blood-vessels. In the central nervous system they are supported and held together, not only by connective tissue, but by a peculiar form of tissue, the neuroglia.

FIG. 42.—MEDULLATED NERVE-FIBERS OF FROG, TREATED WITH SILVER NITRATE SOLUTION. $\times 560$. 1. At γ , node of Ranvier; *a*, axis-cylinder, of which only a small extent is silvered; *b*, biconical swellings displaced downward owing to manipulation. 2 Axis-cylinder with the silvered portion in situ, at α . 3. Axis-cylinder with cross-markings. Techn. No. 31.

II. MICROSCOPIC ANATOMY OF THE ORGANS.

I. THE CIRCULATORY SYSTEM.

THE BLOOD-VESSELS.

The blood-vessels are composed of fibrous connective tissue, elastic fibers, and smooth muscle-fibers, mingled in widely different proportions, and arranged in strata or tunics. In general, a uniform disposition of the elements prevails in each tunic; longitudinal in the inner and outer, circular in the middle tunic. An exception to this occurs in the complicated structure of the heart and in the simple structure of the capillaries.

THE HEART.

The walls of the heart consist of three membranes: 1, the endocardium; 2, the powerfully developed muscular layer; 3, the pericardium.

FIG. 42.—CROSS-SECTION OF PAPILLARY MUSCLE OF HUMAN HEART. *m*, Muscle-fibers in section; *p*, perimysium with small deeply-stained nuclei; *v*, blood-vessels. $\times 240$. Techn. No. 33.

The *endocardium* is a connective-tissue membrane which contains smooth muscle-fibers and numerous elastic fibers. The latter are especially well developed in the auricles, where they form a close-meshed network or are blended in a fenestrated membrane (Fig. 21). The free surface, that directed toward the cavity of the heart, is clothed with a simple layer of irregularly polygonal epithelial (endothelial) cells.

The naked *muscle-fibers*, whose structure has been described, are surrounded by a delicate perimysium, and are united by numerous lateral processes. The arrangement of the muscle-fibers is very intricate. The muscle tissue of the auricles is entirely separate from that of the ventricles. In the former an outer transverse layer, common to both, and an independent longitudinal layer in each can be distinguished. In addition, numerous small bundles pursue independent courses in other directions. The muscle tissue of the ventricles is much more irregularly distributed. The bundles extend in all directions, often describing a figure-of-eight in their course. Between the auricles and ventricles lie firm tendinous ligaments, the *annuli fibrosi*, of which the right is stronger than the left. Similar but less developed ligaments lie at the arterial orifices of the ventricles. Numerous muscle-fibers take their origin in these ligaments.

The *pericardium* is a connective-tissue membrane penetrated by elastic

fibers, clothed on its outer (visceral layer) and inner (parietal layer) surfaces by a single stratum of epithelium. The parietal pericardium is considerably thicker than the visceral. Between the latter and the heart fat-cells are found.

The *valves* of the heart are composed of fibrous connective tissue, continuous with that of the annuli fibrosi, and their surfaces are clothed by the endocardium. Muscle-fibers are found only in the roots or attached edges of the valves. The numerous blood-vessels of the muscular wall of the heart form typical capillary networks with elongated meshes (see the Muscular System). The pericardium and endocardium, the latter in its deeper strata, also possess blood-vessels.

The *lymph-vessels* are extremely numerous in the heart. They form a comprehensive system embracing all the lymph-spaces in the clefts between the muscle-fibers, and accompany the blood-vessels in their course. The nerve supply of the heart includes medullated nerve-fibers derived from the pneumogastric and nonmedullated sympathetic nerve-fibers from the cervical ganglia; along their course numerous ganglion-cells occur.

FIG. 44.—SMALL ARTERIES OF MAN. *i*, Nuclei of intima, the outlines of the cells are invisible; *m*, nuclei of circularly-disposed muscle fibers of media; *a*, nuclei of the adventitia; *A*, artery with the surface in focus; *B*, artery with the lumen in focus, at *m'* the nuclei of the muscle-fibers of the media are seen in optical section; *C*, small artery shortly before transformation into capillaries. The media here consists of a few isolated muscle-fibers. $\times 240$. Techn. No. 34 a.

THE ARTERIES.

The walls of the arteries comprise three coats: 1, tunica intima; 2, tunica media; 3, tunica adventitia. The elements of the tunica media are transversely disposed, those of both the other tunics chiefly longitudinally. The structure and thickness of these coats varies with the size of the artery. This renders their classification as small, medium, and large arteries desirable.

The *small arteries* include the terminal branches shortly before their transformation into capillaries. The *intima* consists of elongated, spindle-shaped epithelial cells and a structureless membrane, the so-called *internal elastic membrane*, which in somewhat larger arteries assumes the character of a fenestrated membrane. The *media* is formed of a single layer of circularly-disposed smooth muscle-fibers. The *adventitia*, the external coat, is composed of lon-

gitudinally-disposed bundles of connective tissue and fine elastic fibers. It blends insensibly with the surrounding connective tissue.

The *arteries of medium size* comprise all the arteries of the body with the exception of the aorta and the pulmonary artery. The *intima* of these vessels has increased in thickness owing to the interposition between the endothelium and internal elastic membrane of delicate fibrous connective tissue, flattened corpuscles, and networks of elastic fibers. This subendothelial layer is absent in the uterine arteries of young individuals, in the coeliac, external iliac, the renal, and the mesenteric arteries. The *media*, in addition to several superimposed layers of circularly-arranged smooth muscle-fibers, comprises wide-

FIG. 45.—PORTION OF CROSS-SECTION OF THE BRACHIAL ARTERY OF MAN. $\times 100$. Techn. No. 33.

meshed networks of elastic fibers. At the inner boundary of the media of some arteries longitudinally-disposed muscle-fibers occur; these are especially well-developed in the subclavian artery. The proportion of the two tissues in the different arteries is extremely variable. In the coeliac, femoral, and radial arteries the muscle tissue preponderates; in the carotid, axillary, and common iliac, the elastic tissue. The *adventitia* has also become stouter. Thick elastic fibers occur in especial profusion at the boundary of the media and in many arteries form a continuous layer designated the *external elastic membrane*. New elements in the adventitia of medium size arteries are smooth muscle-

fibers, which appear in single longitudinally-disposed bundles, and never form a continuous layer.

In the *large arteries* (aorta and pulmonary artery) the epithelial cells of the intima are broader and more polyhedral in outline than in medium-sized vessels. The subepithelial layer consists of fibrous connective tissue, elastic networks, and flattened, stellate, or spherical cells. The elastic network is closer meshed the nearer to the intima it is, and finally passes into a fenestrated membrane corresponding to the internal elastic membrane of small and medium-

FIG. 46.—ENDOTHELIUM OF A MES-ENTERIC ARTERY OF RABBIT. Surface view. $\times 260$. Techn. No. 35.

FIG. 47.—PIECE OF CROSS-SECTION OF THORACIC AORTA OF MAN $\times 100$. Techn. No. 33.

sized arteries. The *media* of the large arteries is characterized by the preponderance of elastic tissue over the muscular elements. The thin elastic networks of the media of medium-sized arteries is here replaced by close networks of broad elastic fibers or by fenestrated membranes, which alternate regularly with the lamellæ of smooth muscle-fibers. The elastic elements, like the muscle-fibers, are circularly arranged. The muscular lamellæ are penetrated in an oblique direction by elastic fibers which connect all the elastic elements of the media. The media of the larger medium-sized arteries possess the elastic

membrane; it is well-marked in the carotids, which closely approach in structure the large arteries. The adventitia of large arteries presents no essential peculiarity and differs but slightly from that of the medium-sized arteries. It does not possess the external elastic membrane. In the lower animals smooth muscle-fibers are present.

THE VEINS.

There is no definite proportion between the size of the veins and the thickness of their walls, and no basis for a division into groups as with the arteries. The veins are characterized by the preponderance of fibrous connective tissue, and the slighter development of the muscular and elastic elements. Three coats, as in the arteries, may be distinguished. Owing to the meager development of the media some histologists have suggested that only two coats are present, the tunica intima and tunica adventitia, and that the layers usually regarded as tunica media belong to the latter. The intima consists of a single

FIG. 48.—PIECE OF CROSS-SECTION THROUGH A VEIN OF LIMB OF MAN. $\times 100$. Techn. No. 33.

layer of endothelial cells which are elongated only in the smallest veins, in others are polygonal in outline. In medium-sized veins, 2 to 9 mm. in diameter, nucleated connective-tissue elements occur in the subendothelial layer, which in large veins (femoral, popliteal, and superior cava) is arranged in distinct lamellæ. Surrounding this is the internal elastic membrane, which is structureless in small veins, but in medium-sized and large veins it is represented by elastic networks. Oblique and longitudinally-disposed smooth muscle-fibers occur in the intima of the iliac, femoral, saphenous, and mesenteric veins.

The media exhibits great variation. It is composed of circular muscle-fibers, elastic networks, and fibrous connective tissue, and is best developed in the veins of the lower extremities (especially in the popliteal), less in the veins of the upper extremities, still less in the large veins of the abdominal cavity, and is absent in many veins (those of the pia and dura, the bones, and the

retina, in the superior cava, and also in the veins proceeding from the capillaries).

The usually well-developed adventitia consists of intercrossing bundles of connective tissue, elastic fibers, and longitudinally-disposed smooth muscle-fibers, which are more highly developed in the veins than in the arteries. The adventitia of certain veins (the trunk of the portal and the renal) possesses an almost complete membrane of longitudinally-arranged muscle-fibers (Fig. 49).

The *valves* of the veins are folds of the intima, covered on both surfaces by epithelial cells, which, on the surface directed toward the vascular stream, are elongated in the direction of the current; on the opposite surface, toward the wall of the veins, they are elongated transversely.

THE CAPILLARIES.

The capillaries establish the communication between the arteries and veins. There are a few exceptions, as for example in the corpora cavernosa. In the transformation of the arteries into capillaries a gradual simplification of the structure of the vessel-wall follows (Fig. 44). The media grows continually thinner, until in the vessels between the smallest arteries and capillaries it is represented by a few isolated circular muscle-fibers, at wide intervals, that ultimately disappear entirely. The adventitia becomes correspondingly attenuated until it consists of a thin layer of connective-tissue fibers and corpuscles that also ultimately vanish, so that at the last the only part of the vessel-wall that remains is the intima. This is also reduced to a stratum of plate-like, nucleated endothelial cells. The walls of the capillaries, therefore, consist of a simple layer of endothelial cells, spindle-shaped, and united at their edges by a small amount of cement-substance.

The capillaries divide without decrease in caliber and by anastomosis with neighboring capillaries form networks differing widely in the size of their meshes. The closest meshes occur in the capillary networks of secretory organs, as in the lung and the liver; wide-meshed networks in the muscles, the serous membranes, and the special sense organs. The reverse obtains with regard to the caliber of the capillaries; the widest capillaries are found in the liver, the narrowest in the retina and in the muscles.

Development of Capillaries.—Only the developmental processes in the post-embryonic epoch will be considered here. A minute conical mass appears on the wall of an existing capillary, resting with a broad base on the latter and having a tapering, sharp-pointed free end. In the further course of development this pointed free end unites with another off-shoot arising from a different point on the capillary wall. They are solid at first but gradually become hol-

FIG. 49.—CROSS-SECTION OF THE RENAL VEIN OF MAN. $\times 50$. Techn. No. 33.

low by an extension of the lumen of the capillary, and subsequently the walls of the new vessels become differentiated to endothelial cells. The development of new capillaries is always consummated in connection with existing capillaries. These blind capillary sprouts may be hollowed out at an early period; corpuscles that flow into them disintegrate because they are excluded from the circulation and the interchange of gases. They fall into fragments, which have been erroneously interpreted as hematoblasts; they have no connection with the true hematoblasts.

All medium and large blood-vessels possess small blood-vessels (*vasa vasorum*) that provide for the nutrition of their walls; they run almost exclusively in the adventitia. The intima is always without blood-vessels.

All blood-vessels are furnished with nerves, which form a plexus of medullated fibers in the media of the arteries and veins. From these, nonmedullated fibers arise which are distributed to the muscle-fibers. The capillaries are

FIG. 50.—SURFACE VIEW OF A PORTION OF THE GREATER OMENTUM OF A SEVEN-DAYS' RABBIT. *c*, Blood capillaries, some containing blood-corpuscles; *s*, capillary sprout tapering to a free solid point; *i*, young capillary, the greater part of which is hollow; at *s'* still solid; *k*, nuclei of peritoneal endothelium. $\times 240$. Techn. No. 37.

accompanied by encircling networks of delicate nonmedullated nerve-fibers. Many blood-vessels are surrounded by lymph channels; occasionally the lymph-spaces in the adventitia unite to form a complete ensheathing sinus, the adventitial or perivascular lymph-space.

The *carotid gland* is really no gland but consists essentially of blood-vessels. The capillaries arising from the division of the single arterial vessel differ greatly in width, and are surrounded by numerous cells resembling the plasma-cells of connective tissue, arranged in rounded groups forming the so-called secondary nodules. The veins are collected at the periphery of the organ, which besides contains fibrous connective tissue, isolated ganglion-cells, and a conspicuous number of medullated and nonmedullated nerve-fibers. Similar in structure is the *coccygeal gland*, the blood-vessels of which are characterized by hemispherical evaginations.

THE BLOOD.

The blood is a slightly clammy, red-colored liquid which consists of a fluid substance, the *blood-plasma*, and formed elements, the *blood-cells*, the blood-platelets, and elementary granules. The blood-cells are of two kinds: colored blood-cells and colorless blood-cells.

The *colored* blood-cells are soft, flexible, highly-elastic elements, and possess smooth, slippery surfaces. In man and in other mammals they have the form of a flat-circular disk, slightly concave on each surface, and therefore resemble biconcave lenses. (Exceptions occur in the llama and the camel, in which the colored blood-cells are oval.) The average diameter in man is $7.5\ \mu$, the thickness $1.5\ \mu$. The colored blood-corpuscles of domesticated mammals are all smaller, the largest are those of the dog ($7.3\ \mu$). The colored blood-cells consist of a *stroma* (protoplasm), the spaces of which are filled with the blood-coloring matter, the *hemoglobin*. The hemoglobin imparts to the corpuscle its yellow or yellowish-green color. A nucleus and a proper cell-mem-

FIG. 51.—BLOOD-CORPUSCLES MAGNIFIED 560 TIMES. *A.* Of man: 1-6. Discoidal colored blood-cells: 1, seen with close focus; 2, with distant focus; 3 and 4, viewed edgewise; 5, crenated in consequence of evaporation; 6, after treatment with water; 7, spherical colored blood-corpuscle; 8, colorless blood-corpuscle; 9, blood-platelets. *B.* Of frog: 10-13. Colored blood-cells: 10, fresh nucleus, indistinct; 11, a few minutes later, nucleus plainly visible; 12, seen from the side; 13, after treatment with water; 14, living; 15, dead colorless blood-corpuscle. Techn. Nos. 38-41.

brane are wanting. The colored blood-corpuscles of fishes, amphibians, reptiles, and birds are distinguished from those of mammals by their oval, biconvex form, their generally greater size ($22\ \mu$ long by $15\ \mu$ broad in the frog), as well as by the presence of a round or oval nucleus; in other respects they exhibit the same properties as those of mammals.

The *white or colorless blood-cells* (leucocytes) occur not only in the blood but also in the lymphatic vessels, where they are termed lymph-corpuscles. They are also found outside of the vessels, in bone-marrow, in adenoid tissue, in fibrous connective tissue, and also between epithelial and gland-cells, where they have wandered by their power of amoeboid movement, and are therefore described as "wandering cells."

The colorless blood-cells consist of a clammy protoplasm and a nucleus, and are without a cell-membrane. A definite form cannot be described, because during life they are engaged in amoeboid activity. In a condition of rest they are spherical (Fig. 52).

Their size and the properties of the nucleus and protoplasm have led to the following classification :

1. The smallest leucocytes, measuring 4 to 7.5 μ . They possess a proportionately large round nucleus surrounded by a narrow zone of protoplasm, so small in amount that it can scarcely be demonstrated by the usual methods (Fig. 52, *a*). These are regarded as young forms; they exhibit little activity and are found chiefly in adenoid tissue.

2. The second kind have a diameter of 7.8 to 10 μ ; their nucleus is spherical and surrounded by a larger amount of granular protoplasm. The nucleus may be cleft or lobulated (Fig. 52, *b*). Occasionally several disjoined nuclei are present; the slender filaments uniting the several parts of the lobulated nucleus are frequently overlooked, which then simulates several separate nuclei. The latter form is very active; the lobulation of the nucleus is in fact the expression of this activity; seventy-seven per cent. of the leucocytes of the blood are of this form.

3. The third kind of leucocytes measure from 8 to 14 μ , and are characterized by their granular protoplasm; the granules are variable in quantity and react differently to stains. According to their affinity for acid, basic, or neutral dyes, oxyphile, basophile, and neutrophile leucocytes are distinguished.*

FIG. 52.—COLORLESS BLOOD-CELLS OF MAN. *a*, Cell with neutrophile granules. $\times 600$, Techn. No. 39.

The determination of the proportionate number of, as well as the ratio between, the colored and colorless blood-corpuscles is coupled with considerable difficulty, and only approximately-correct estimates can be given. In man one cubic millimeter of blood contains about 5,000,000 colored corpuscles. The white blood-corpuscles are present in the blood in much smaller number, about 1 to 300 to 500 colored.

The *blood-platelets* are very unstable, colorless, round or oval disks having a diameter one-third to one-fourth less than that of the colored blood-cells; at times they are present in the blood in large numbers. A leading rôle in the process of coagulation of the blood is ascribed to them.

The *elementary granules* are for the most part fatty granules transferred from the chyle to the blood. They are frequently observed in the blood of the lower mammals, but are not normally present in the blood of man.

After death, or as a result of changes within the vessel-walls, the blood, under the influence of two substances which pass into solution in the plasma, fibrinoplastin and fibrinogen, coagulates, and fibrin is formed. The coagulated blood separates into two parts, the clot and the serum. The clot is red, and contains all the colored and the majority of the colorless blood-corpuscles and the fibrin, which microscopically consists of a feltwork of fine, straight,

* Ehrlich, who made this classification, proceeds therein from a different standpoint than that of the chemist; acid colors are those, for example, with which acids develop the coloring principle.

interlacing filaments. Chemically fibrin resembles glutinous connective tissue. The supernatant serum is colorless and contains a few colorless blood-cells.

The coloring substance contained in the colored corpuscles, the *hemoglobin*, crystallizes under certain conditions, and in nearly all vertebrates the crystals belong to the rhombic system. Their form in the different animals varies greatly; in man it is usually prismatic. Hemoglobin is readily decomposed. One of the decomposition products is hematin, which yields hematinoidin and hemin. Crystals of hematinoidin occur within the body in old extravasated blood, for example, in the corpus luteum, and are rhombic prisms of orange-red color. The hemin crystals, when well-developed, are rhombic plates or needles of a mahogany-brown color. They are often very irregular in form, and as a positive indication of the presence of blood have an important legal relation.

Development of Colored Corpuscles.—From the earliest period of embryonic development and during the whole of life nucleated colored blood-cells (hematoblasts, erythroblasts) are found in certain situations. Their number fluctuates and runs parallel to the energy of the blood-forming processes. By indi-

FIG. 53.—1. Hemin crystals of man; whetstone forms on the right. 2. Crystals of common salt. 3. Hematinoidin crystals of man, magnified 560 times. 4. Hemoglobin crystals of the dog, magnified 100 times; a, a crystal showing a tendency to fall apart lengthwise. Techn. No. 44.

rect division they give rise to the nonnucleated colored blood-corpuscles, which at first contain a nucleus, but lose it later. As centers for the formation of blood in the embryo the liver and, later, the spleen, in the adult exclusively the bone-marrow, may be mentioned.

2. THE LYMPHATIC SYSTEM.

THE LYMPH-VESSELS.

The walls of the larger lymph-vessels, from 0.8 to 0.2 mm., like the blood-vessels, are composed of three coats. The intima consists of endothelial cells and a network of delicate elastic fibers with elongated meshes. The media is formed of circularly-disposed smooth muscle-fibers and a few elastic fibers. The walls of the smallest lymph-vessels and the lymph-capillaries are composed exclusively of extremely delicate endothelial cells, often having sinuous outlines. The lymph-capillaries, unlike the blood-vessels, present at frequent intervals constrictions and dilatations, and where they branch are often considerably widened; the networks they form are more irregular.

The question as to the origin of the lymph-vessels is not yet satisfactorily determined; while some authors are of the opinion that the lymph-capillaries form a closed system, according to another view, widely entertained, the lymph-capillaries are open toward the periphery, and are in direct connection with the system of intercommunicating cell-spaces of connective tissue. These interfascicular clefts are by some set apart as "lymph canaliculi" from the lymph-vessels with well-defined walls composed of continuous layers of cells; other authors include the lymph-canaliculi in the lymph-vessels.

According to the first opinion the surplus of nutritive fluids (tissue-juices) diffused through the walls of the blood-capillaries, and not used in the nutrition of the tissues, is returned to the closed lymph-capillaries by endosmosis; the second view holds that the tissue-juices pass directly from the tissue into the patent orifices of the lymph-capillaries.

FIG. 54.—LYMPHATIC VESSEL OF THE MESENTERY OF RABBIT, showing the boundaries of the endothelial cells. $\times 50$. Techn. No. 35.

It is a significant fact that the lymph-vessels of the pleura and of the peritoneum are in open communication with their respective cavities through small openings—*stomata*—between the endothelial cells, which in the pleura are found at the intercostal spaces, and in the peritoneum on the central tendon of the diaphragm.

THE LYMPH-NODES.

The lymph-nodes (incorrectly "lymph-glands") are macroscopic, encapsuled bodies found along the course of the lymph-vessels; they are usually rounded, oval, or flat, kidney-shaped structures, and differ greatly in size. At one side there is often a scar-like depression, the *hilus*, at which the efferent lymph-vessels emerge. The afferent lymph-vessels penetrate the nodes at various points. Their construction becomes intelligible if we proceed from the following conception: in certain localities three to six lymph-vessels divide and anastomose, forming a kind of rete mirabile, and then reunite into the same or a less number of usually narrower lymph-vessels.* The dividing lymph-vessels are called afferent (*vasa afferentia*), the reunited, efferent vessels (*vasa efferentia*). Within the meshes of this reticulum lie spherical and elongated masses that consist of adenoid tissue. The spherical masses, the *secondary nodules* (follicles), occupy the periphery; the elongated masses, the *medullary cords*, the center of the lymph-node.

* *Retia mirabilia* were first described in connection with the blood-vessels. They occur along the course of both arteries and veins; the vessel suddenly breaks up into branches and these into capillaries, which reunite into a single vessel. Exquisite examples of such networks occur as the glomeruli of the kidneys.

The node is enveloped in a capsule of fibrous connective tissue, which sends into the interior of the organ stout fibrous bundles, the *trabeculae* (Fig. 55). Finer extensions from the trabeculae form a reticulum which breaks through the walls of the lymph-vessels, penetrates the secondary nodules and the medullary cords, and forms a support for the numerous leucocytes present.

The lymph-nodes consist of a *cortical* and a *medullary* region that vary greatly in their proportionate extent. The cortex contains the secondary nodules, which are directed toward the center of the organ and merge into the medullary cords (Fig. 55). The secondary nodules and medullary cords are surrounded by the sinus-like continuations of the afferent lymph-vessels. The latter, in this situation, are greatly expanded and are termed *lymph-sinuses*; they are interwoven with the connective-tissue reticulum. The lymph-vessels never penetrate the interior of the secondary nodules. The secondary nodules and the medullary cords are composed of *adenoid* tissue; that is, of a reticulum of connective tissue the meshes of which are crowded with leucocytes. In many of the secondary nodules there is a light, rounded area, the *germinal center*, in

FIG. 55.—SECTION OF A LYMPHATIC NODULE OF A NINE-DAYS'-OLD CAT. $\times 30$. Techn. No. 47.

which karyokinetic figures are always to be found. Multiplication of cells also occurs in the medullary cords, but in a much slighter degree. The secondary nodules are, therefore, centers for the formation of the leucocytes, which pass into the lymph-sinuses and thence into the vasa efferentia.

The capsule consists of fibrous connective tissue and smooth muscle-fibers, which in the large lymph-nodes of some animals are arranged in bundles. The trabeculae have the same structure; they pass between the secondary nodes and medullary cords but do not come into contact with them, being separated from them by the lymph-sinuses. The walls of the lymph-sinuses are formed of a simple layer of plate-like cells; similar cells clothe the surface of the nodules and the cords, and are applied to the trabeculae and the connective-tissue reticulum.

The structure of the lymph-nodes is difficult to recognize, owing to several complications. These consist in: 1, the merging of neighboring secondary nodules; 2, the anastomosis of the medullary cords in the form of a coarse network; 3, the network formed by the trabeculae; 4, the interlacing of

the networks formed by the medullary cords and the trabeculae; 5, the presence of leucocytes in the lymph-sinuses, which must be removed by special methods. The secondary nodules, the medullary cords, and the leucocytes in the lymph-sinuses form a soft mass, the *pulp* or parenchyma of the lymph-node.

The majority of the blood-vessels enter at the hilus, the others at various points on the surface of the node. The latter are smaller vessels and divide in the capsule and in the large trabeculae, in the axes of which they run. The large artery entering at the hilus divides into a number of branches, which are surrounded by a richly-developed connective tissue. The branches are principally distributed to the adenoid tissue, only a few entering the trabeculae; they pass through the lymph-sinuses, to the medulla and to the cortex, and in both situations break up into rich capillary networks, which supply the oxygen needed in the formation of leucocytes. The veins emerge at the hilus.

FIG. 56.—FROM A SECTION THROUGH THE MEDULLA OF A LYMPHATIC NODULE OF OX. $\times 50$. In the upper half the trabeculae and medullary cords are cut lengthwise, in the lower half crosswise. Both form an anastomosing network. In the lymph-sinuses fine fibers of reticular connective tissue are seen, which still in part contain leucocytes. Drawn with change of focus. Techn. No. 46.

The nerves are few in number, the supply including bundles containing both medullated and nonmedullated fibers; their ultimate distribution is still undetermined.

THE PERIPHERAL LYMPH-NODULES.

Adenoid tissue is not confined to the lymph-nodes, but, in different degrees of development, occurs widely distributed in many mucous membranes; sometimes as *diffuse*, sometimes as *definitely-circumscribed* infiltrations of leucocytes. These formations are not included in the lymphatic system. More highly-specialized structures, nodules with germinal centers, resembling exactly the secondary nodules of the lymph-nodes, are also found in the mucous membranes; these are termed the *peripheral lymph-nodes*, and included in the lymphatic system. In many mucous membranes they occur isolated, as the *solitary nodules* (solitary follicles), or grouped, as "Peyer's patches," and lie always in a simple layer in the membrana propria close to the epithelium (see the

Digestive Organs). The number and distribution of the peripheral lymph-nodes is subject to considerable fluctuation, not only in the different species of animals, but in different individuals; since their mass varies and there are frequent transitions from circumscribed to diffuse infiltration, it is probable that they are temporary structures, that arise and disappear during life. The follicles are distinguished from the real lymph-nodes by the absence of the encircling lymph-sinus. The only exceptions occur in the rabbit, in which the sinus is present in the Peyer's patches, but not in the solitary follicles. But the possession of a germinal center, a brooding-place for young leucocytes, appears in so far to entitle them to a place in the lymphatic system. The young leucocytes only in part enter the lymph-vessels; many wander through the epithelium to the surface of the mucous membrane.

THE LYMPH.

The lymph is a colorless fluid in which leucocytes (lymph-corpuscles) and granules are suspended. The latter are immeasurably small, consist of fat, and are found principally in the lymph- (or chyle-) vessels (lacteals) of the intestine; frequently they are present in enormous numbers; they impart the white color to the chyle. In other lymph-vessels the fatty granules occur but sparingly.

THE SPLEEN.

The spleen is a "blood-vessel gland" consisting of a connective-tissue *capsule* and of a soft red mass, the *spleen pulp*, which is composed of blood-vessels and adenoid tissue.

The *capsule* is invested by a reflection of the peritoneum, with which it is firmly united. It is composed of dense fibrous connective tissue and a network of elastic fibers. In some animals (the dog, cat, pig, etc.), but not in man, smooth muscle-fibers are also present. Numerous cylindrical or band-like prolongations, the *trabeculae*, extend into the interior of the organ, where they form a framework in the spaces of which lies the spleen pulp. In the lower mammals the trabeculae also contain smooth muscle-fibers. At the hilum of the spleen the capsule furnishes special sheaths for the blood-vessels—adventitial sheaths—which blend with the adventitia of the latter and accompany them for long distances. The sheaths of the arteries are the seat of numerous leucocytes, which form a continuous envelope along the entire course of the

FIG. 57.—FROM A CROSS-SECTION OF HUMAN SPLEEN, showing well-developed Malpighian corpuscles, each pierced eccentrically by an artery. The right branch of the artery has a continuous sheath of adenoid tissue. $\times 10$. Techn. No. 50.

vessel, as in the guinea-pig, or, as in man, the cat, etc., are limited to certain points where they form spherical masses, 0.2 to 0.7 mm. in size, the so-called *Malpighian corpuscles*. Between these many intermediate forms exist, as in the mouse and rabbit.

FIG. 58.—ELEMENTS OF HUMAN SPLEEN. $\times 560$. 1. Colorless blood cells. 2. Epithelial cells. 3. Colored blood-corpuscles. 4. Cells containing granules: the upper one enclosing also a blood-corpuscle. 5. Techn. No. 49.

FIG. 59.—RETICULAR CONNECTIVE TISSUE OF HUMAN SPLEEN. $\times 560$. Drawn from the edge of a shaken preparation. Techn. No. 51.

FIG. 60.—THREE KARYOMITOTIC FIGURES FROM A SECTION THROUGH SPLEEN OF DOG. $\times 560$. The filaments not visible with this magnification. Techn. No. 52.

The Malpighian corpuscles are usually situated in the forks of the smaller arteries, and in such wise that the artery pierces them through the center or near the periphery. In their minute structure they agree entirely with the secondary nodules of the lymph-nodes, and occasionally even contain germinal centers.

FIG. 61.—SECTION OF SPLEEN OF MOUSE, MAGNIFIED 85 TIMES, showing the nerves supplying the wall of an artery. The sheath of the artery is infiltrated its entire length by lymphoid cells. The boundary between the pulp and the artery is indicated by a dotted line. Techn. No. 54.

The Malpighian corpuscles are also temporary structures, continually disintegrating and developing anew.

The spleen pulp forms a network of cords which, like that of the lymph-nodes, occupies the interstices of the trabecular framework. Occasionally

the cords are attached to the Malpighian corpuscles. The spleen pulp is composed of a delicate connective-tissue reticulum and numerous cellular elements.

FIG. 62, A.—SECTION THROUGH AN INJECTED SPLEEN OF CAT. Techn. No. 53.

The latter are in part leucocytes, in part slightly larger nucleated cells; also cells containing colored blood-corpuscles and free colored blood-corpuscles. Pigment-granules are also present.

FIG. 62, B.—SCHEMATIC DRAWING OF SECTION 62, A.

The Blood-vessels.—The arteries of the spleen give off branches to the trabeculæ and to the pulp, and contribute to the dense capillary network of

the Malpighian corpuscles. The veins proceed from a wide-meshed network of capillaries (venous spaces, venous capillaries) occupying the intervals between the trabeculæ and the pulp cords (Fig. 62). The larger veins run alongside the arteries. The precise mode of communication between the arteries and the veins is not yet determined. The arteries break up into slender capillaries which do not anastomose with one another. According to one view, these arterial capillaries are directly continuous with the "venous" capillaries, and the blood-vessels are closed on all sides. Other authors hold that the arterial capillaries pass into spaces without definite walls, "intermediate lacunæ," which connect with veins with perforated sieve-like coats, and that the latter establish the communication with the veins with closed walls.

The superficial lymphatics on the surface of the spleen, numerous in the lower mammals, are scantily developed in man. The deep lymphatics in the interior of the spleen are also few in number; the exact relations of the latter have not yet been fully investigated.

The nerves, which comprise a few medullated fibers and many naked axis-cylinders, follow the course of the trunks and branches of the arteries, supplying the muscular coats of the latter, and in the lower mammals the smooth muscle-fibers of the trabeculæ (Fig. 61). Plexuses of nonmedullated nerve-fibers occur in the spleen pulp, partly sensory in their nature, and probably proceed from the branches of the medullated nerve-fibers just mentioned.

III. THE ORGANS OF THE SKELETAL SYSTEM.

The skeletal system consists of a large number of hard parts, the bones, which are joined together by special structures and form in their entirety the skeleton.

In the embryo the greater part of the skeleton consists of cartilage, which in the course of development is supplanted by bone, and with the exception of a few remnants disappears; such remnants are the costal cartilages and the cartilages of the joints, which cover the opposed surfaces of many bones. Skeletal cartilages are also found in the respiratory passages and the organs of special sense.

THE BONES.

On sawing through a fresh long bone, it will be seen at once that its texture is not everywhere alike, but that the osseous tissue appears in two forms: the one, a dense, firm, apparently structureless substance, constitutes the principal portion of the periphery and is termed *compact bone* (substantia compacta); the other, toward the axial cavity, appears as an irregular

reticulum of thin osseous lamellæ and slender trabeculæ, and is called *spongy bone* (*substantia spongiosa*). The interstices of the spongy bone, as well as the central marrow-cavity, are filled by a soft mass, the *bone-marrow*; the surface of the bone is enveloped in a fibrous membrane, the *periosteum*. The proportion between the compact and the spongy substance is different in the short bones, which consist chiefly of the latter, the compact substance being limited to a narrow zone at the periphery. Flat bones have sometimes thicker, sometimes thinner outer shells or crusts of compact substance, while the interior is filled with spongy substance. In the epiphyses of the long bones, as in the short bones, the spongy substance preponderates.

FIG. 63.—PIECE OF A LONGITUDINAL SECTION THROUGH HUMAN METACARPUS. 30. Fat-drops are seen in the Haversian canals. At x Haversian canals open on the outer, at xx on the inner surface of the bone. Techn. No. 57.

The *spongy substance* consists entirely of osseous tissue; the compact substance, on the other hand, contains besides the bone canaliculi and lacunæ, a second system of coarser channels, 22 to 110 μ wide, which divide dichotomously and form a wide-meshed network. These channels contain the blood-vessels and are named *Haversian canals*. In the long bones, in the ribs, the clavicle, and the inferior maxilla their course is parallel to the long axis of the bone; in short bones they run mainly in one direction, for example, vertically in the vertebrae; in the flat bones their course is parallel to the surface, not infrequently along lines that radiate from a point, as in the tuberosity of the parietal bone. The Haversian canals open on the outer surface of the bone, as well as on the inner surface, directed toward the *substantia spongiosa*.

The ground-substance or matrix of bone is arranged in lamellæ. The fibrillæ are joined in bundles, and these placed side by side form the lamellæ.

According to the disposition of these strata three lamellar systems may be distinguished: an annular or *Haversian system*, in which in cross-sections eight to fifteen lamellæ are seen to be concentrically arranged around an Haversian canal; these lamellæ are called *Haversian* or *special lamellæ* (Fig. 64). Between the Haversian systems, which come into contact only here and there, are irregularly-disposed lamellæ, the *interstitial* or *ground lamellæ*; these are connected with the third lamellar system, the *circumferential* or *fundamental lamellæ*, in which the osseous strata encircle the outer and inner free surfaces of the bone. The circumferential lamellæ contain a variable number of canals, which, unlike the Haversian canals, are not the centers of annular systems of lamellæ; they are called Volkmann's canals, and contain the "perforating vessels." The latter frequently connect with the vessels of the Haversian canals; the passage of the Volkmann's canals into the latter is a gradual one.

The bone *lacunæ* in the compact substance extend in a definite direction. In the Haversian systems their long axis is parallel to the long axis of the

FIG. 64.—PIECE OF CROSS-SECTION OF METACARPUS OF MAN. $\times 50$. The Haversian canals, *h*, still contain marrow (fat-cells). Techn. No. 57.

Haversian canals, and they are bent so that cut transversely in the cross-section of an Haversian system they appear concentrically curved. In the interstitial lamellæ the lacunæ are placed irregularly; in the circumferential lamellæ so that their surfaces extend parallel to the surfaces of the lamellæ. The bone canaliculi open into the Haversian canals, and also on the free outer and inner surfaces of the bone.

The bone-marrow occupies the axial cavity of the tubular bones, fills the interstices of the spongy substance, and is also found in the larger Haversian canals. It is of a red or yellow color, and therefore two varieties are recognized—the *red marrow* and the *yellow marrow*. The red marrow is found in the vertebræ, the bones of the skull, the sternum, and the ribs—in all young bones (also in the long bones of some animals); the yellow marrow occurs in the short and the long bones of the extremities. In old and in sick persons the marrow is mucoid and reddish-yellow, and is characterized by its poverty in fat.

The elements of red marrow comprise a delicate connective-tissue reticulum which supports a few fat-cells, larger and smaller marrow-cells, and giant-cells (myeloplaxes). In the larger marrow spaces the connective tissue forms a membrane, the endosteum, which lines the free surface. The marrow-cells exhibit forms resembling leucocytes; the giant-cells are structural anomalies representing leucocytes enlarged and altered in form; they are huge,

FIG. 65.—ELEMENTS OF HUMAN BONE-MARROW. $\times 600$; 1-5. Various forms of bone-cells. 6. Eosinophilous cell. Techn. No. 57 b.

extremely irregular, multinucleated masses of protoplasm. The shape of the nucleus varies greatly; it may be round, lobulated, band- or hoop-shaped, or it may fashion a network. A uninuclear giant-cell may become multinuclear through the division of the nucleus by constriction, or a corresponding part of the protoplasm may be set free with the nucleus and the result is a uninuclear cell. The view interpreting these indications of division as the phenomena of

FIG. 66.—PIECE OF CROSS-SECTION OF FEMUR OF ADULT MAN. $\times 80$. Techn. No. 56. The lamellæ can be recognized by the disposition of the lacunæ.

a reversed series of processes—the merging of several cells into one—has very little probability, since the process of budding has been observed in living cells. There are also found in the red marrow nucleated cells with yellow-colored protoplasm like that of the colored blood-corpuscles; these are the hematoblasts (erythroblasts) (Fig. 65). Yellow pigment-granules that appear in the different cells are regarded as the remains of disintegrated colored blood-corpuscles.

The yellow marrow consists of a connective-tissue reticulum containing much fat. Marrow-cells and hematoblasts in yellow marrow are found only in the head of the humerus and the femur.

The periosteum is a compact connective-tissue membrane, in which two layers can be distinguished. The outer is characterized by its richness in blood-vessels and forms the connection with adjacent structures, tendons, fasciæ, etc.; the inner layer contains few blood-vessels, but is rich in elastic fibers and spherical or spindle-shaped connective-tissue cells. At places on the inner surface a layer of cubical elements may be found, which are of importance in the development of the bone. The periosteum is sometimes firmly, sometimes loosely attached to the bone; the attachment is secured by the blood

FIG. 67.—VERTICAL SECTION THROUGH HEAD OF A METACARPUS OF ADULT MAN. $\times 50$. Techn. No. 59.

vessels passing to and from the bone and by Sharpey's fibers, which pierce the circumferential and interstitial lamellæ and extend in all directions (Fig. 66).

The blood-vessels of the bone, the marrow, and the periosteum are in the closest connection with one another, and also with surrounding structures. Small branches (not capillaries) of the numerous arteries and veins of the periosteum enter the Haversian and Volkmann's canals, and on the inner surface of the bone are in communication with the blood-vessels of the marrow. The latter is supplied by the nutrient artery, which in its course through the compact substance gives off branches to the same, and in the marrow breaks up into a rich capillary network. The veins that take up the capillaries of the

marrow have no valves. Lymph-vessels with well-defined walls occur only in the superficial layer of the periosteum.

The nerves are numerous and consist partly of medullated, partly of gray fibers. They enter the Haversian canals and are distributed to the bone-marrow and the periosteum, and in the latter occasionally terminate in Pacinian corpuscles.

THE ARTICULATIONS OF BONES.

Two forms of articulations are recognized: synarthroses, joints characterized by immobility; diarthroses, joints in which the bones are movable, one upon the other.

In *synarthroses* the bones are joined either by ligaments, the union constituting a *syndesmosis*; or by the intervention of cartilage, forming a *synchondrosis*.

The ligaments are fibrous bands possessing a structure like that of tendon, or they are composed of elastic tissue. The latter are distinguished by the possession of numerous robust elastic fibers which are never arranged in bundles or lamellæ, but are always separated by loose connective tissue. The ligamentum nuchæ, ligamenta subflava, and ligamentum stylohyoideum are elastic ligaments.

The sutures also belong to the syndesmoses; short fibrous ligaments extend from one serrated osseous edge to the other.

The cartilage in *synchondroses* is rarely only of the hyaline variety, but usually is in part fibro-cartilage (especially at the borders in contact with the bone) and in part hyaline, in which the cell capsules are frequently calcified.

The intervertebral ligaments, which belong to the *synchondroses*, possess a soft, jelly-like center, the "gelatinous nucleus," which contains groups of cartilage cells; it is the remains of the notochord, the embryonic precursor of the vertebral column. At the periphery of the intervertebral ligaments there is a narrow tendinous zone.

In *diarthroses* the parts entering into a joint are the articular ends of the bones, the capsular ligament, the marginal fibro-cartilages, and the inter-articular cartilages.

The articular ends of the bones are from 0.2-5 mm. thick and are covered by a stratum of cartilage thinning out at the edges. The superficial cartilage cells are flattened and placed parallel to the surface; those in the median plane are rounded and are often collected in groups; in the deepest plane, the groups of cells are partly arranged in longitudinal rows, vertical to the surface of the cartilage; adjoining this, but separated by a narrow striated belt, is a small zone of calcified cartilage interposed between and connecting the hyaline cartilage with the osseous tissue (Fig. 67). Not all the articular cartilages exhibit the same structure; the cartilages of the costo-vertebral, the sterno-clavicular, the acromio-clavicular, and the maxillary articulations, and the head of the ulna are not hyaline, but fibro-cartilage; the distal articular surface of the radius is covered with dense fibrous tissue.

The glenoid ligament and the interarticular cartilages do not exhibit the characteristic cartilage matrix, but consist of a compact fibrous connective tissue and of spherical cells. To the same category belong the so-called sesamoid cartilages; that of the tendon of the peroneus longus, however, contains genuine cartilage.

In the adult, nerves and blood-vessels are wanting in the articular cartilages, as also in the interarticular cartilages and the glenoid cartilage.

The capsular ligaments consist of an external fibrous membrane, the *fibrous capsule*, of varying thickness, possessing a structure like that of the ligaments, and of an internal membrane, the *synovial membrane*, the free inner surface of which is smooth and glossy; the outer layer of the latter is composed of loose elastic fibers and fibrillar tissue containing fat-cells; within this is a thin lamella of parallel connective-tissue bundles, in which, toward the interior, there are spherical or stellate nucleated cells, 11–17 μ in size; they are not numerous except at points subjected to great pressure, where they occur in large numbers and form an endothelial membrane three or four strata thick.

FIG. 68.—SYNOVIAL VILLI WITH BLOOD-VESSELS FROM HUMAN KNEE-JOINT. Magnified 50 times. The epithelium has fallen from the apex of the left villus, exposing the connective tissue. Techn. No. 60.

The synovial membranes often send free processes containing fat into the synovial cavity, and bear on their free surfaces the *synovial fringes* or *villi*, variously shaped processes, mostly of microscopic size, which closely beset the edges of the joint surfaces, and bestow upon the synovial membrane a reddish, velvety appearance. They consist of connective tissue and are clothed by a single or a double layer of cells.

The larger blood-vessels of the synovial membranes lie in the loose connective-tissue layer; the capillaries extend through the inner thin connective-tissue stratum and form vascular tufts in the villi. Some of the villi are without vessels. The lymph-vessels lie close under the endothelium.

The nerves run in the loose connective tissue and terminate in part in Pacinian corpuscles.

The *synovia* consists principally of water; it contains only six per cent. of solids and no formed elements.

THE CARTILAGES.

The costal cartilages are of the hyaline variety; the matrix exhibits the usual characteristics; the cells frequently contain fat. The surface is enveloped by a compact fibrous membrane, the perichondrium, which consists of interlacing fibrous bundles and elastic fibers.

The articular cartilages are covered by the perichondrium only at their edges, not on their free surface. Where the cartilage and the perichondrium are in contact there is a gradual transition of the one tissue into the other, and consequently the attachment between the two is very firm.

The perichondrium carries the nerves and the blood-vessels; the latter also run in canals within growing cartilage. In the adult, cartilage is devoid of blood-vessels; the nutrition of the tissue depends upon diffusion from adjoining structures. The costal cartilages in advanced life are often extensively ossified and contain blood-vessels.

The cartilages of the special-sense and the respiratory organs will be described in the corresponding chapters.

FIG. 69.—FROM A DORSO-PLANTAR LONGITUDINAL SECTION OF GREAT TOE OF FOUR-MONTHS' HUMAN EMBRYO. Two-thirds of the first phalanx represented. $\times 50$. 1. Lacunæ enlarged and containing many cartilage-cells. The cells cannot be distinguished with this magnification, only their nuclei, which appear as minute dots. At 2, developing cartilage; cells in groups of three and four, each group produced by repeated division from one cartilage-cell. Techn. No. 61.

DEVELOPMENT OF BONE.

The bones are comparatively late structures in their appearance. The development of the muscles, nerves, blood-vessels, brain, spinal cord, etc., is already well advanced in the embryo at a time when not a trace of bone is present. At that period the skeleton is formed of hyaline cartilage. With the exception of certain parts of the cranium and nearly all the bones of the face, the entire skeleton is mapped out in cartilage. In the upper extremity, for example, the humerus, radius, ulna, carpus, and the skeletal parts of the hand, consist of cartilaginous pieces, which, however, are not hollow like the bones by which they are subsequently replaced, but are solid throughout. The

osseous skeleton then gradually appears in the place of the cartilaginous. All the osseous parts that in the embryo were preceded by cartilage are called *primary* or *endochondral* bone: all other bones, not preformed in cartilage, *secondary* or *intermembranous* bone.

The primary bones include all the bones of the trunk and extremity, the greater part of the cranium (the occipital bone with the exception of the upper portion of the tabular part, the sphenoid with the exception of the internal pterygoid plate, the temporal bone with the exception of the squamous portion and the annulus tympanicus, the ossicles of the ear, the ethmoid bone, the inferior turbinal), and the hyoid.

The secondary bone includes the bones forming the sides and vertex of the cranium and nearly all the bones of the face.

FIG. 70.—FROM A DORSO-PALMAR LONGITUDINAL SECTION OF FINGER OF FOUR-MONTHS' HUMAN EMBRYO. Two-thirds of second phalanx represented. $\times 50$. The calcified trabeculae are covered by a thin layer of endochondral bone. (More highly magnified in Fig. 71.) Techn. No. 61.

DEVELOPMENT OF PRIMARY BONE.

Two modes of bone formation are here to be considered: 1, *endochondral ossification*, formation of osseous tissue within the cartilage, and 2, *periosteal* (better *perichondral*) *ossification*, formation of osseous tissue immediately surrounding and also on the cartilage. The phylogenetically older perichondral ossification usually begins earlier, but for didactic reasons will be described subsequently to the process of endochondral formation.

1. ENDOCHONDRAL OSSIFICATION.—The first indications of this process consist in changes at certain places within the cartilage; the cells enlarge and divide, so that several lie in one lacuna; a deposition of lime-salts takes place within the matrix, in consequence of which it becomes granular and dull—it

calcifies. Such places may be recognized with the unaided eye, and are called *centers of ossification* (better, centers of calcification). The portions of the cartilage remote from the centers of calcification continue to grow in

FIG. 71.—FROM A LONGITUDINAL SECTION OF PHALANX OF FIRST FINGER OF FOUR-MONTHS' HUMAN EMBRYO, $\times 220$. In the endochondral bone irregular lacunae with bone-corpuscles may be seen. Techn. No. 61.

thickness and length, while at the center growth ceases, and consequently the cartilage at this point appears constricted (Fig. 69). Meanwhile, on the surface of the center of calcification, a tissue rich in blood-vessels and young

cells—*osteogenic tissue*,* has made its appearance. This penetrates into the cartilage, the calcified matrix is absorbed, the cartilage-cells are set free and disintegrate; a little space—the *primary marrow-cavity*—is excavated in the center of calcification.

These processes are repeated in the immediately surrounding cartilage; that is, the matrix calcifies, the cartilage-cells enlarge, new portions of the cartilage break down, and as a result the primary marrow-space is gradually and continuously extended. At the same time the capsules of many cartilage-cells are opened, the cells degenerate, and the intervening calcified matrix projects into the marrow-space in the form of irregular processes or trabeculæ.

FIG. 72.—CROSS SECTION OF THE UPPER HALF OF DIAPHYSIS OF HUMERUS OF FOUR-MONTHS' HUMAN EMBryo. *h*, Developing Haversian spaces; *h'*, blood-vessel. $\times 35$. Techn. No. 61.

The primary marrow-cavity is now filled with blood-vessels and young cells. The fate of these cells in the further course of development varies. They retain their original form and become marrow-cells, or they become fat-cells, or—most important—they become bone-forming cells, *osteoblasts*. In the latter event, a number of cells arrange themselves in a single layer on the walls of the marrow-cavity and on the surface of the calcified trabeculæ, and produce the matrix of true osseous tissue.

As a result of this activity, the trabeculæ and the walls of the marrow-

* This is not a good name, inasmuch as the tissue has not originated from bone, but is to become bone.

cavity are soon covered with a thin layer of bone-substance, which gradually increases in thickness. Thus step by step the former solid cartilage is transformed into spongy bone, the trabeculae of which still contain a residue of calcified matrix (Fig. 72).

2. PERICHONDRAL OSSIFICATION.—This mode of bone formation is also accomplished through the agency of the osteoblasts derived from the osteogenetic tissue at the surface of the center of calcification; they form strata of spongy osseous tissue on the surface of the cartilage, which is distinguished from the endochondral bone in the absence of remnants of calcified cartilaginous matrix, because the perichondral bone is formed at the circumference and not in the interior of the cartilage. The formation of the first Haversian canals may be observed in the perichondral bone (Fig. 72). The latter is not formed in a continuous layer of uniform thickness, but at frequent intervals depressions or recesses may be observed containing blood-vessels surrounded by osteoblasts (Fig. 72 *hh*); at first the recesses are open toward the periphery, but with the advancing development of the osseous strata they are closed in, and then represent Haversian canals. The osteoblasts enclosed within the canals produce new osseous strata, the Haversian lamellae.

FIG. 73.—FROM A CROSS-SECTION OF THE LOWER JAW OF A NEWBORN DOG. $\times 240$. Metaplastic type. Techn. No. 61.

By the absorption of the cartilage and its substitution by osseous tissue, also by the deposition of bone-substance on its exterior, the piece of cartilage has become a bone.

The essence of the foregoing processes consists in an absorption of the parts of the primordial skeleton and in the new formation of the same by the development of bone-substance. This mode of bone formation is termed *neoplastic* in contradistinction to the rarer *metaplastic* mode, in which the cartilage is not destroyed but ossified, and the cartilage matrix becomes the bone matrix, the cartilage-cells the bone-cells (as for example, in the angle of the inferior maxilla) (Fig. 73).

SECONDARY OR INTERMEMBRANOUS BONE.

In this the fundament on which the formation of bone occurs is not cartilage but connective tissue. Isolated bundles of connective tissue calcify; on these osteoblasts derived from embryonal cells arrange themselves and produce bone, in the manner above described (Fig. 74). The intermembranous

bone is enclosed on all sides by connective tissue: when osseous tissue is in direct contact on one side with cartilage, without the intervention of connective tissue, the resulting formation is not intermembranous, but perichondral bone.

GROWTH OF BONE.—In *tubular bones* ossification in the diaphysis begins much earlier than in the epiphyses (in the humerus the center of ossification in the diaphysis appears in the eighth fetal week, in the epiphyses in the first year of life); blood-vessels grow into the calcified cartilage which is transformed at first by endochondral, later by perichondral, formation into bone. The articular surfaces of the bone remain permanently cartilaginous; and a narrow zone between the diaphysis and each epiphysis, the *epiphyseal cartilage*, persists until the growth of the bone is completed. An active growth of cartilage is maintained here, which

FIG. 74.—FROM A HORIZONTAL SECTION OF PARIETAL BONE OF HUMAN EMBRYO. $\times 240$. Techn. No. 61.

by extension of the primary marrow-cavities of the diaphysis and the epiphyses is continually being supplanted by bone. In this way the bone grows in length. Increase in thickness takes place by the "apposition" of new periosteal strata.

In the *short bones* ossification takes place, as in the epiphyses, at first by endochondral formation; after the absorption of the superficial remnant of cartilage, a perichondral osseous shell is formed.

In the *flat bones* perichondral precedes endochondral formation.

Intermembranous bones grow in superficies and thickness by the formation of new osseous masses at their edges and their surfaces respectively. As a consequence of the abundant deposition of bone-substance on the surface, the outer and the inner tables of compact bone are formed, which enclose between them spongy bone; the latter is termed *diploë* in this situation. The osseous masses at first possess a coarse-fibered, and later (from about the first year of life) a fine-fibered matrix.

RESORPTION OF BONE.—Immediately following the initial formation of osseous tissue a contrary process, *resorption*, becomes perceptible, by which the calcified cartilage matrix and many parts of the primary or endochondral bone are removed. Resorption is actively carried on in the tubular bones in the formation of the marrow-spaces (in a lesser degree in other bones) and on the surface of bones until their typical form is completed. The femur of a three-year-old child, for example,

Giant-cells lying in Howship's lacunæ.

FIG. 75.—FROM A CROSS-SECTION OF HUMERUS OF A NEWBORN CAT. $\times 240$. H, Haversian space, containing two blood-vessels and marrow-cells. Techn. No. 61.

contains scarcely any of the osseous tissue present at birth. In the interior of the compact bone irregular excavations may be seen, the so-called Haversian spaces, formed by the absorption of the innermost Haversian lamellæ, which, however, may be partly filled again by the deposition of new osseous substance.

Wherever resorption of bone takes place, multinucleated giant-cells may be seen lying in depressions or pits—*Howship's lacunæ*—which they have excavated in the bone. In this situation the giant-cells bear the name of *osteoclasts* (Fig. 75).

Even in the fully-developed skeleton the processes of apposition and resorption still occur at isolated places.

IV. THE ORGANS OF THE MUSCULAR SYSTEM.

The muscular system is composed of a large number of contractile organs, the muscles, which consist of cross-striated muscle-tissue and are joined to the skeleton, the skin, the viscera, etc., by the intervention of special connective-tissue formations, the *tendons*, and accessory parts of similar structure, the *fasciæ*, *tendon-sheaths*, and *bursæ*.

Each *muscle* is composed of striated muscle-fibers which, as a rule, are disposed parallel and lengthwise in bundles surrounded by a connective-tissue sheath, the *perimysium*.

Interlacing is rare, but occurs, for example, in the tongue. Neighboring muscle-fibers are never in direct contact, but each individual fiber is enveloped in a delicate connective-tissue sheath, the *endomysium*, which is joined to neighboring sheaths (Fig. 76). A number of muscle bundles form a muscle, the surface of which is covered by a robust connective-tissue membrane, the *epimysium*. The several sheaths are connected with one another.

The grouping of the primary bundles into secondary bundles, which in a certain number of instances are grouped into tertiary bundles, and finally united to form a muscle, is an arbitrary division, and in many preparations cannot be recognized.

FIG. 76.—FROM A CROSS-SECTION OF THE ADDUCTOR MUSCLE OF A RABBIT. *P*, Perimysium, containing two blood-vessels, at *g*, *m*, muscle-fibers; many are shrunken and between them the endomysium, *p*, can be seen; at *x* the section of muscle-fiber has fallen out. $\times 60$. Techn. No. 62.

The perimysium is composed of fibrillar connective tissue and numerous fine elastic fibers, and occasionally contains fat-cells; it conveys the nerves and lymph-vessels. The endomysium contains only capillaries and terminal branches of nerves.

The post-embryonal increase in the thickness of the muscle depends less on the division than on the growth in thickness of the already existing muscle-fibers.

The *tendons* are characterized by the parallel course of their fibers, their firm union, and the scarcity of elastic fibers. They are composed of bundles of fibrous tissue, the primary or tendon-bundles, which are held together by looser connective tissue and form secondary bundles. Each primary bundle consists of a number of parallel fibrillæ, running a straight course and united by a small amount of cement-substance. Between the primary bundles lie the

FIG. 77.—A. FROM A CROSS-SECTION OF DRIED TENDON OF ADULT MAN. $\times 50$. Techn. No. 63. B. FROM a cross-section of tendon fixed with chromic acid (adult man). Techn. No. 64.

cellular elements of the tendon, fusiform, stellate, polygonal, or flat cells, arranged in longitudinal rows. They partially clasp the primary bundles and unite with one another by means of processes. Elastic fibers are found chiefly in the loose connective tissue; in the dense tendon-bundles they are scarce and occur in the form of a fine wide-meshed network.

The union of muscles with tendons and fibrous membranes is effected by the extension of the endomysium of the muscle-fiber to these structures, and the blending of the tissues; the sarcolemma takes no part in this, but as a continuous sheath, with pointed or obliquely blunted ends, closely invests the muscle-fibers. When the muscle-fibers are spread out in a membrane they attach themselves to the connective tissue by pointed or forked ends.

The *fasciæ* in part exhibit the same structure as the tendons, and in part they are fibrous membranes richly provided with elastic fibers. The latter is

the case where they form sheaths for the muscles and do not furnish surfaces for the attachment of the muscle-fibers.

The *tendon-sheaths* and the *bursæ* consist of a layer of connective tissue and elastic fibers, varying in thickness, the inner surface of which is covered patch-wise by polygonal endothelial cells. Where the endothelium is wanting the connective tissue is dense and rich in rounded elements resembling cartilage-cells. The majority of the tendon-sheaths have small vascular processes exactly like the synovial fringes.

The blood-vessels of striated muscles are very numerous and uniformly distributed; the capillaries are among the thinnest in the human body, and form networks characterized by elongated rectangular meshes, closely surrounding the individual fibers.

FIG. 78.—TENDONS FROM RAT'S TAIL. $\times 240$. A. Tendon-cell viewed in profile, B, from the surface. At X the nucleus is bent so that it is seen partly in profile (the shaded portion) and partly from the surface (the light portion). Techn. No. 65.

FIG. 79.—FROM A SAGITTAL LONGITUDINAL SECTION OF THE GASTROCNEMIUS OF FROG. $\times 50$. The uppermost transverse line represents the endomysium seen from the surface. Techn. No. 66.

The veins are provided with valves throughout their course, even in their smallest branches. The lymph-vessels are few in number and follow the branches of the smaller blood-vessels.

For the nerves, partly sensory and partly motor, see the *Peripheral Nerve-Endings*.

The blood-vessels of the tendons and the thinner fasciæ are very scarce, and run in the loose tissue between the fibrous bundles; the tendon-sheaths, on the other hand, and the bursæ have a rich vascular supply. Lymph-vessels are found only on the surface of the tendon.

The medullated nerves of tendons terminate in part in a close plexus of gray nerve-fibers, and in part in *tendon-spindles*, a formation resembling the motorial end-plates. End-bulbs and Pacinian corpuscles also are found in tendons, fasciæ, and tendon-sheaths.

V. THE ORGANS OF THE NERVOUS SYSTEM.

I. THE CENTRAL NERVOUS SYSTEM.*

THE SPINAL CORD.

Topography.—The spinal cord consists of a white and a gray substance, distinguishable by the unaided eye. The arrangement and the relation of these two substances are best recognized in cross-sections of the spinal cord.

The *white substance* encircles the gray matter, and is partially divided by a deep anterior cleft, the *anterior median fissure*, and a posterior *septum* (formerly called the posterior median fissure) into a right and a left half. Each half is subdivided by the furrows marking the exit of the anterior and the posterior roots of the spinal nerves into a large *lateral column*, an *anterior column*, and a *posterior column*. In the lower cervical and the upper thoracic regions two divisions may be distinguished in the posterior column, of which the median portion is named the *column of Goll* (funiculus gracilis), and the lateral portion the *column of Burdach* (funiculus cuneatus). The anterior columns are united by the *white commissure* at the bottom of the anterior median fissure.

The *gray substance* appears in cross-section in the form of an H, and consists of two lateral columns or masses connected by a horizontal bridge, the *gray commissure*. On each mass thick *anterior cornua* and slender *posterior cornua* may be distinguished. Adjoining the lateral portions of the anterior horns, and horizontally even with the central canal, are the *lateral cornua*, which are especially well-developed in the upper thoracic region. From the front boundary of the anterior cornua, the *anterior roots* of the *spinal nerves* emerge in several bundles, while the *posterior roots* enter at the postero-median side of the posterior cornua. At the base of the posterior cornua, laterally, a net-like mass of gray substance, the *reticular process*, is found; at the median side, near the gray commissure, lies the well-defined *column of Clark* (dorsal nucleus), visible in the whole length of the thoracic and in the upper part of the lumbar regions of the cord; and capping the summit, a glistening, jelly-

*I shall confine myself here to a brief account of the topography and histology of the spinal cord and the brain. An exhaustive presentation of the architecture of the central nervous system, the paths of the nerve-fibers, and the complicated origins of the cranial nerves in the "nuclei" of the oblongata would exceed the limits of this "Histology." The student is referred to special text-books, of which Edinger's "Vorlesungen über den Bau der nervösen Centralorgane" is recommended.

like mass, the *substantia gelatinosa Rolandi* may be distinguished. Posteriorly to this is the small *zona spongiosa*, at the dorsal edge of which is found the *zona terminalis*, an area of cross-sectioned thin nerve-fibers. In the gray commissure lies, in section, the *central canal*, which extends through the whole length of the cord and is surrounded by the *substantia gelatinosa centralis*. The central canal is from 0.5 to 1 mm. in diameter; not infrequently it is impervious. The portions of the gray commissure in front of and behind the canal are named respectively the *anterior* and the *posterior gray commissure*. From all points of the periphery of the gray substance coarser or finer processes, the *septula medullaria*, radiate into the white substance. In the cervical and

FIG. 80.—CROSS-SECTION OF THE CERVICAL ENLARGEMENT OF THE HUMAN SPINAL CORD. $\times 7$.
Techn. No. 68.

the lumbar enlargements of the cord the gray matter is more powerfully developed than in the thoracic region, and there is a corresponding variation in the form of the H. The end of the cord, the *conus medullaris*, consists almost wholly of gray substance.

Minute Structure.—The *gray substance* will be first considered, a knowledge of its composition being essential to the comprehension of the structure of the white substance. It consists of multipolar ganglion-cells, which with their ramifying and axis-cylinder processes form a dense nervous tangle, the “nerve-felt,” which is penetrated by nerve-fibers proceeding in part from the white columns and in part from the posterior roots, and the whole is supported by a framework of *neuroglia*.

The *nerve-cells* are divided in accordance with the relations of their axis-cylinder processes into *motor-cells* and "column-cells" (*Strangzellen*).

The *motor nerve-cells* lie in two groups in the anterior horn, an antero-median and postero-lateral, separate in the cervical and lumbar enlargements, but in the uppermost cervical and in the thoracic regions united in a single cluster (Fig. 80). In longitudinal sections it may be seen (conspicuously in amphibians) that the cell groups, governed by the origin of the single roots, have a correspondingly typical segmental arrangement. The cells possess a large cell-body (67 to 135 μ) and long protoplasmic processes, dendrites, extending far into the surrounding substance; the nerve- or axis-cylinder process emerges from the summit of the anterior cornu, makes an oblique descent through the white substance, at the same time receives a medullary sheath, and becomes the axis-cylinder of a medullated nerve-fiber. Occasionally the axis-cylinder process gives off a few lateral twigs before leaving the

FIG. 81.—CROSS-SECTION OF THE SPINAL CORD OF A SEVEN-DAYS' OLD EMBRYO CHICK. $\times 80$. The white matter is but slightly developed, the central canal still very large. Techn. No. 70.

gray matter. It leaves the spinal cord as a part of the anterior root-fiber bundle. All anterior root-fibers arise from the motor-cells of the anterior horn, and on the same, not the opposite, side.

The *column-cells* constitute the chief mass of the nerve-cells of the gray substance, and lie partly scattered, partly grouped in the lateral horn and in the dorsal nucleus. The majority are smaller than the motor nerve-cells and possess fewer and less-branched, but far-reaching, dendrites. Their axis-cylinder process, after sending off numerous collateral fibrils in the gray substance, enters the white substance—the anterior or the lateral columns, very rarely the posterior—either on the same or on the opposite side; in the latter case the cells are sometimes termed commissure-cells, because the axis-cylinder passes through the anterior gray commissure before entering the white substance. The commissure-cells occupy an area embracing the central canal in an arch on the ventral side. Having arrived in the white substance, the axis-

cylinder process of the majority of the column-cells divides into a vertical ascending and descending "stem-fiber," which in its course parallel to the longitudinal axis of the spinal cord sends off lateral twigs (collateral fibrils), which return to the gray substance, where they terminate in tufts of free fibrils; the stem-fibers themselves finally terminate like the collateral fibers. The collateral fibers that enter into the anterior columns are tolerably robust and penetrate the anterior cornua singly or in bundles, where they weave a net around the large motor-cells; they are especially robust in the antero-lateral

FIG. 82.—SCHEME OF THE SPINAL CORD SHOWING THE LOCATION AND RAMIFICATION OF THE NERVE-CELLS, ALSO OF THE POSTERIOR NERVE-ROOTS.

curve of the anterior horn. Less numerous are the collateral fibers coming from the lateral columns, which go chiefly to the substantia gelatinosa centralis, and only those ventral to the substantia gelatinosa Rolandi are well developed; the latter pass to the opposite side and form the *dorsal* or *posterior commissure*. In the adult, all the nerve-processes of the column-cells are enveloped in a medullary sheath. The axis-cylinder processes coming from the vesicular column of Clarke do not divide in the white substance, but turn cranialward and proceed to the cerebellum. The axis-cylinder processes of

still other column-cells, arrived in the white substance, turn, without dividing, upward or downward. There have been described, under the name of "pluricordonal" cells, elements whose axis-cylinder processes divide into two or three branches and continue in as many fibers in the different columns.

The cells so far described belong to the nerve-cells of the first type (Deiters's). There is another kind of cell, whose nerve-process rapidly divides, but remains within the gray substance. For this reason these elements are termed *interior cells*; they occur in the posterior columns and are nerve-cells of the second type (Fig. 84).

The *nerve-fibers* that enter the anterior and lateral columns arise in part from the medullated collateral fibers and the ends of the nerve-processes of the column-cells, in part from the axis-cylinder processes (likewise invested by a medullary sheath) that come from the brain.* To these belong the medullated

FIG. 83.—FROM A LONGITUDINAL SECTION OF THE SPINAL CORD OF A NEWBORN RAT. X 110. The section shows two posterior nerve-roots. The collateral fibrils are not visible. Techn. No. 70.

fibers of the posterior roots which originate in the centripetal processes of the cells of the spinal ganglia. These posterior root-fibers enter the spinal cord in two groups, a lateral which runs in the zona terminalis, and a median which runs in the posterior columns. The fibers do not enter the gray substance directly, but each first divides Y-shape into an ascending and a descending stem-fiber (Fig. 83), from which numerous collateral fibers diverge at right angles; these now enter the gray substance, and with their tufts of terminal fibrils distribute themselves over nearly every point of the same: one set terminates principally in the summit of the posterior horn; these take their origin in the lateral root-fiber group and form a very fine-fibered dense plexus, that partly lies in the substantia gelatinosa Rolandi (Fig. 84 c); the second set terminates in Clarke's column (Fig. 84 a); they originate in the median root-fiber group, as also a third set, which passes through the middle of the substantia gelatinosa Rolandi ventralward into the anterior cornu, and there radiates fan-shape and surrounds the motor-cells in a network of fibrils (Fig. 84 b); the latter set forms the reflex bundle. This and the collateral fibers of Clarke's column sink into the gray substance in a curve with the concavity lateralward, and form a conspicuous mass easily perceived. There are other collateral fibers which pass through the posterior gray commissure to the fibers (the so-called "crossed") of the opposite side. The stem-fibers, probably only after a long course (several centimeters),

* For an account of the exact course of these fibers the student is referred to special textbooks.

turn into the gray substance, where they terminate like the collateral fibrils in fine branches.

The peculiarities of the *substantia gelatinosa centralis* and *Rolandi*, which belong to the gray substance, are dependent upon the abundance of neuroglia, and will be described with this.

The *white substance* is composed entirely of medullated nerve-fibers which are without the sheath of Schwann. The fibers differ greatly in thickness; the thickest are found in the anterior columns and in the lateral parts of the posterior columns; the thinnest in the median part of the posterior columns and in the lateral columns where the white and the gray substances touch. In the remaining portions thick and thin fibers are intermingled. The majority of

FIG. 84.—CROSS-SECTION OF THE SPINAL CORD OF A NEWBORN RAT SHOWING COLLATERAL FIBRILS. $\times 75$. In the right half only one representative of each kind has been sketched. Techn. No. 70.

the nerve-fibers run parallel with the long axis of the spinal cord, and hence in cross-sections are cut transversely. In addition there are fibers that take an oblique direction; these are found in large numbers in front of the gray commissure, where they cross at acute angles and form the white commissure (Fig. 80).

An attempt to classify the nerve-fibers according to their origin will result as follows: 1, fibers which are continuations of the posterior root; the entire posterior column consists of posterior root-fibers, because the latter (or their stem-fibers), entering in the lumbar region, are pushed toward the median line by the fibers entering at higher levels; 2, fibers which are continuations of the column-cells; 3, fibers which are continuations of the ganglion-cells of

the brain. Both the latter occupy the anterior and the lateral columns and are united in compact bundles (funiculi).

The *supporting framework* of the spinal cord is constructed of two genetically distinct formations: 1, *fibrous connective-tissue* extensions of the pia, which penetrate the white substance as sheaths for the blood-vessels; this mesenchymal framework grows constantly thinner as it approaches the gray substance, into which it does not extend; 2, the *neuroglia* ("nerve-cement") which is derived from the same embryonic anlage as the central nervous system. The neuroglia consists principally of nucleated elements, the *glia-cells*, and, possibly, of a small amount of homogeneous ground-substance. There are two kinds of glia-cells. The one kind are the *ependymal cells*, which in a

FIG. 85.—GLIA-CELLS FROM THE SPINAL CORD. $\times 280$. Techn. No. 70.

single layer line the lumen of the central canal. In youth they are beset with cilia. Their cylindrical bodies are prolonged in an extended process which in the embryo reaches to the surface of the spinal cord, where it terminates in a simple or branched end (Fig. 85). The cells of the ependyma are phylogenetically the older; they arise also ontogenetically first, but in the further course of development undergo retrogression in different degrees, which not infrequently leads to complete obliteration of the central canal. The second kind of glia-cells are the so-called *Deiters's cells*, which in the beginning of their development lie in the gray substance; later they retreat into the white substance, and then assume various shapes. Of the numerous processes of these cells one, the "chief process," originates first; the others, partly fine and partly coarser "secondary" processes, later. Many of these cells, with their

much-branched processes, reach to the surface of the spinal cord, where they terminate in expanded ends and form a distinct border or *glia-zone*. Two varieties of the developed cells, united by many transitional forms, may be distinguished: the “mossy-cells” and the “spider-cells.” The mossy-cells possess shorter, very richly-branched processes, and not infrequently are applied to the blood-vessels; they occur chiefly in the gray substance; the spider-cells have a small cell-body from which, besides short, also many longer, rigid, less-branched processes radiate; these occur chiefly in the white substance and are not apt to be confused with the ganglion-cells. By the interlacing of the numerous fine processes of neighboring *glia*-cells (they do not anastomose) a close web is constructed which envelops each individual nerve-fiber.

In the gelatinous substance of the central canal and the posterior cornua the neuroglia assumes a totally different appearance. In the former the Deiters's cells, with their (in this situation) very long, stiff, undivided processes are concentrically arranged in a fiber-wreath (Fig. 85). These and the cells of the ependyma are also together called “central ependyma filaments.” The *substantia gelatinosa Rolandi*, in addition to the small ganglion-cells and the nerve-fibers (collateral fibers) traversing it, consists of a granular substance—a transformation of the numerous and very delicate processes of Deiters's cells occurring here.

THE BRAIN.

The brain, like the spinal cord, is composed of a white and a gray matter, which in their minute structure agree on the whole with the same substances in the cord. But the arrangement of the two substances in the brain is a much more diversified one than in the spinal cord.

The gray substance of the brain occurs in four aggregations: 1, as the *cerebral cortex*, the outer sheet covering the surface of the cerebral hemispheres; 2, in the form of discrete masses in the cerebral ganglia,—the *corpora striata*, the *optic thalami*, the *corpora quadrigemina*; 3, as the lining of the *ventricles*, which is the direct continuation of the gray substance of the spinal cord; 4, as the *cerebellar cortex*, the sheet covering the surface of the cerebellum.

Discrete masses also occur in the interior of the cerebellum. All these aggregations have numerous connections with one another by means of fiber-tracts.

FIG. 86.—FROM A CROSS-SECTION OF THE HUMAN SPINAL CORD IN THE REGION OF THE LATERAL COLUMN. $\times 180$. Techn. No. 69.

FIG. 87.—VERTICAL SECTION OF HUMAN CEREBRAL CORTX. $\times 60$. Techn. No. 71.

FIG. 88.—SCHEME OF CEREBRAL CORTX, sketched from specimens prepared according to Techn. No. 73 b. 1. Cell of Cajal. 2, 2'. Small pyramidal cells. 3. Large pyramidal cell. 4. Polymorphous cell. 5, 5'. Cells of the second type. 6. Nerve-fiber ending in the superficial zone; a, mossy-cell; b, spider-cell.

THE CEREBRUM.

In vertical sections of the cerebral cortex four zones, not sharply defined from one another, may be distinguished:—

1. The *molecular layer* (neuroglia layer), the most superficial, in ordinary preparations appears finely granular or reticulated, and contains, besides a few cells, an interlacement of medullated nerve-fibers running horizontally, the *tangential fibers* (Fig. 87). By means of Golgi's method, it may be seen that the reticulum is formed in part by the dendrites of the pyramidal cells (of the second and third zones) and in part by the processes of glia-cells. Besides the latter there are in the molecular zone the cells of Cajal; these possess an irregularly-shaped cell-body and processes running parallel to the surface, from which ascending lateral twigs diverge (Fig. 88, 1). In the lower animals four or more processes have been described; in man these cells have only been observed in the embryo, and evidence of the nerve-processes was not obtainable. The nervous nature of Cajal's cell is, therefore, not yet determined.

2. The *zone of the small pyramidal cells* (Fig. 87 and 88) is characterized by ganglion-cells 10 to 12 μ in size and of a pyramidal form; the apex of the pyramid is prolonged into a long ramifying protoplasmic process, which after giving off minute lateral twigs enters the molecular zone, where it terminates in numerous, often serrulate, branches (Fig. 88, 2); smaller dendrites spring from the lateral and inferior surfaces of the cell. The axis-cylinder process proceeds from the base and after giving off branched collateral fibers passes, as a rule, toward the white substance to become the axis-cylinder of one or, by division, of two nerve-fibers; occasionally, however, it bends and runs to the molecular layer, where it divides and enters the web formed by the tangential fibers (Fig. 88, 2'). The nerve-processes and also the collateral fibers are enveloped in a medullated sheath. The size of these cells is difficult to determine because of the extension of the cell-body into the apical process.

3. The *zone of the large pyramidal cells* is distinguished from the second zone by the greater size of its elements (20 to 30 μ); the robust axis-cylinder process, after giving off in the gray substance several collateral fibrils, always goes to the white substance (Fig. 88, 3).

4. In the *layer of the polymorphous nerve-cells* the majority of the elements are oval or polygonal; an apical dendrite is wanting, but the delicate nerve-process, after sending off a number of lateral twigs, enters the white substance, where it passes into one, or, dividing into T-branches, into two nerve-fibers (Fig. 88, 4).

FIG. 89.—PYRAMIDAL CELL FROM A PERPENDICULAR SECTION OF THE CEREBRAL CORTEX OF ADULT MAN. $\times 120$. The terminal branches of the dendrites running toward the molecular layer are not visible. Techn. No. 73 b.

In the last three zones ganglion-cells of the second type are also found. Their branching axis-cylinder process is either confined to the gray matter in the vicinity of the cell, or extends to the molecular zone, where after rapid branching it terminates (Fig. 88, 5, 5').

The last two zones both contain numerous medullated nerve-fibers arranged in part in thick "radiating" bundles, which split up into single fibers near the zone of the small pyramidal cells (Fig. 87). The bundles are formed by the descending medullated nerve-processes of the large and small pyramidal cells, and by thick medullated nerve-fibers of unknown source, that ascend from the white substance toward the cortex (Fig. 88, 6), where they divide repeatedly and form the "superradial" and the tangential interlacement (Fig. 88), and finally end in free branches; another set of medullated nerve-fibers runs transversely to the radiating bundles and forms the interradianal reticulum,

FIG. 90.—FROM SECTIONS OF BRAIN OF ADULT MAN. $\times 280$. Techn. No. 73 b.

which is somewhat condensed toward the "superradial" reticulum, and represents the *stripes* of *Gennari* or *Baillarger* (Fig. 87). This and the interradianal reticulum are composed of the medullated collateral fibrils of the nerve-processes of the pyramidal cells.

The structure of the cerebral cortex is modified in certain localities. In the hippocampal and uncinat convolutions the tangential fibers are present in large numbers and form a net-like extended white layer, the *substantia reticularis alba*. In the vicinity of the calcarine fissure the stripes of *Gennari* are developed into the *bundle* of *Vicq d' Azyr*, which may be seen by the unaided eye. Greater or lesser deviations occur in many other localities, which render a division of the above description much more difficult.

Finally, extensions of the pia that penetrate the cerebral cortex in company with the blood-vessels participate in its construction, as also the *neuroglia*, which like that of the spinal cord consists of *ependymal* cells and *Deiters's* cells.

In the embryo the peripheral processes of the former extend to the free surface. Of the latter two varieties are distinguished, as in the spinal cord: the spider-cells, which occur chiefly in the white substance, and the mossy-cells, which are found mainly in the gray substance, where they are in intimate relation with the blood-vessels, to the walls of which they are often attached by one thick process (Fig. 90). On the surface of the cerebral cortex there is a glia-zone composed essentially of the processes of the glia-cells.

THE CEREBRAL GANGLIA.

The gray substance of the cerebral or basal ganglia consists of ganglion-cells varying in size, medullated nerve-fibers, and neuroglia. Macroscopical variations in color depend on the proportions in which the ganglion-cells and nerve-fibers are mingled: wealth of ganglion-cells is rendered perceptible by a dark red-brown color, profusion of nerve-fibers by a pale yellow-gray color.

THE GRAY SUBSTANCE OF THE VENTRICLES.

The gray substance extends from the floor of the fourth ventricle through the aqueduct of Sylvius into the third ventricle, to the tuber cinereum and the infundibulum. It is of especial interest as the place of origin of the cranial nerves. It is composed of neuroglia, nerve-fibers, and ganglion-cells; the majority of the latter are multipolar, and in certain localities are distinguished by their size (as in the nucleus of the hypoglossal nerve), or by their peculiar form (as the spherical ganglion-cells in the upper pair of the corpora quadrigemina).

An extension of the neuroglia and ependyma lining the central canal of the spinal cord lines the floor of the fourth ventricle, the aqueduct of Sylvius, the inner surface of the third and the lateral ventricles; it is composed of similar elements. The columnar or cubical cells of the ependyma of the ventricles in the newborn, and in part also in the adult, possess cilia.

THE CEREBELLUM.

The cerebellum consists of a cortical layer of gray substance composed of three well-defined strata, of which the outer and the inner are macroscopically, the middle, on the contrary, only microscopically perceptible: they are from within outward, the *granule layer*, the *layer of the cells of Purkinje*, and the *molecular layer*.

The *granule layer*, the innermost, is characterized by its rust color and consists of numerous strata of small cells, which by the ordinary methods ex-

FIG. 91.—PIECE OF A PERPENDICULAR SECTION THROUGH THE CORTX OF THE CEREBELLUM OF ADULT MAN. $\times 50$. Techn. No. 72.

hibit a proportionately large nucleus and a small amount of protoplasm. By the aid of Golgi's method it becomes apparent that, apart from the glia-cells, two kinds of ganglion-cells are present: *small granule-cells* and *large granule-cells* (Fig. 92 and 94, 1). The former are multipolar ganglion-cells with short protoplasmic processes, with claw-like endings, and a delicate nerve-process, without a medullary sheath, which passes vertically into the outermost or molecular layer; there it divides into longitudinal T-branches running parallel to the surface and terminating in free unbranched ends. The small granule-cells are the

FIG. 92.—SMALL GRANULE-CELL WITH A PIECE OF THE NERVE-PROCESS, N, AND SHORT DENDRITES, D. From a section through the cortex of the cerebellum of a six-weeks-old cat. $\times 400$. Techn. No. 74.

FIG. 93.—LARGE GRANULE-CELL FROM A SECTION THROUGH THE CORTEX OF THE CEREBELLUM OF A SIX-WEEKS-OLD CAT. $\times 200$. Techn. No. 74.

principal elements of the granule-layer. Less numerous are the *large granule-cells*, multipolar ganglion-cells more than twice the size of the smaller elements, whose ramifying protoplasmic processes extend into the outermost layer, and whose nerve-process, running in the opposite direction, rapidly divides and terminates in a rich ramification penetrating within the granule-layer (Fig. 93 and 94, 2).

A dense plexus of medullated nerve-fibers occurs in the granule-layer; the greater part of the fibers come from the white substance of the cerebellum, and

at the boundary of the granule and middle layers they form a horizontal band, transverse to the longitudinal axis of the convolution, from which fibers run into the molecular layer. A small portion of this plexus is formed by the medullated nerve-processes of the cells of Purkinje (Fig. 94, 3, 3', 3'').

The *middle stratum* of the cerebellar cortex consists of a simple layer of very large multipolar ganglion-cells, the *cells of Purkinje*. Their somewhat pear-shaped bodies send two robust protoplasmic processes into the molecular layer, where they terminate in an uncommonly rich arborization extending to

FIG. 94.—SCHEME OF THE CEREBELLAR CORTEX, sketched from a specimen prepared according to Techn. No. 74. 1. Small granule-cells. 2. Large granule-cells. 3. Network of nerve fibers; 3', horizontal bundles; 3'', fibers of the molecular layer. 4. Cell of Purkinje. 5. Basket-cell. 6. Small cortical cells. Glia-cells: a, of the molecular layer; b, mossy-cell; c, spider-cell.

the free surface (Fig. 94, 4). The ramification does not extend in all direction, but only in planes transverse to the long axis of the convolution. The entire ramification can, therefore, only be seen in sections at right angles to the long axis of the convolution. From the opposite pole of the cell the axis-cylinder process proceeds, which soon acquires a medullated sheath and, passing through the granule-layer, enters the white substance of the cerebellum; while still within the granule-layer it sends off collateral fibrils, which branch and, in part, run back between the cells of Purkinje (Fig. 94).

The *molecular-layer* is distinguished by its gray color and contains two kinds of multipolar ganglion-cells: the *large cortical cells* or *basket-cells* and the small *cortical cells*. The large cortical cells lie in the deeper half of the molecular-layer; their protoplasmic processes extend mainly toward the surface. Their longer nerve-process runs horizontally near the inner margin of the molecular-layer, transversely to the axis of the convolution, sends toward the surface a few collateral fibrils, and in the deeper portions of the layer gives off at successive intervals delicate branches whose terminal ramifications form a basket-like network—*fiber-basket*—around the bodies of Purkinje's cells (Fig. 95). The "basket" often also embraces the beginning of the axis-cylinder process of Purkinje's cell. Nearer to the surface lie the *small cortical cells* (Fig. 96); their nerve-process is difficult to find, and consequently has been but little investigated.

FIG. 95.—BASKET-CELL, FROM A SECTION THROUGH THE CEREBELLAR CORTEX OF A SIX-WEEKS'-OLD CAT. $\times 240$. The five cells of Purkinje were not blackened, but plainly visible; only the outlines of their bodies are sketched. Techn. No. 74.

The medullated nerve-fibers in the molecular-layer are extensions of the reticulum of the granule-layer, and pass in part to the surface, where after losing the medullary sheath they terminate in free branches between the arborization of the protoplasmic processes of the cells of Purkinje, and in part they run horizontally between the bodies of these cells, parallel to the axis of the convolution.

The *neuroglia* of the cerebellum consists of two kinds of cells: the one kind lie at the boundary of the granule-layer, and have small bodies which send a few short processes inward, but many long processes in a straight course toward the free surface, where they terminate in a triangular expansion (Fig. 97, left), and form in this way a relatively thick peripheral glia-layer; the other kind, stellate cells, resemble the mossy-cells of the cerebral cortex (Fig. 97, right); they occur in all the strata. In the white substance typical spider-cells are found.

So long as the cerebellum is not fully developed it is characterized by a series of peculiarities which are wanting in the adult. In embryos and young animals there is over the as yet slightly-developed molecular-layer a superficial granule stratum; the structures in the granule-layer described under the name of "moss-fibers" are developmental forms of medullated nerve-fibers; and of like significance are the "climbing plexuses" found in the environs of the ramifying protoplasmic processes of the cells of Purkinje.

The union of the elements of the cerebellum—as everywhere in the central nervous system—is by contact, not by direct connection.

FIG. 96.—FROM A SECTION OF THE CEREBELLAR CORTX OF ADULT MAN. $\times 240$. The transverse lines are nerve-processes of basket-cells. The cell of Purkinje and the glia-cell are drawn from another part of the specimen for the purpose of demonstrating the difference in size. Techn. No. 74.

The *white substance*—the medulla—of the cerebrum and of the cerebellum, apart from the elements of the supporting framework (connective tissue and neuroglia), consists throughout of medullated nerve-fibers without a neurilemma and varying in thickness from 2.5 to 7 μ .

The *hypophysis cerebri* (pituitary body) is composed of two genetically different parts: (1) a *small posterior lobe* that belongs to the brain, its stalk the infundibulum; it however contains but few nerve-fibers, and consists principally of connective tissue, blood-vessels, and cells which closely resemble bipolar or multipolar ganglion-cells; (2) an *anterior larger lobe* derived as a

diverticulum from the primary oral cavity; it contains tubular acini embedded in loose vascular connective tissue, the majority of which are solid and filled with pale or dark cubical epithelial cells (Fig. 98). Only a few of the acini at the edge of the smaller lobe are hollow; occasionally they contain colloid substance resembling that in the tubules of the thyroid body.

FIG. 97.—TWO GLIA-CELLS FROM A SECTION THROUGH THE CEREBELLAR CORTEX OF ADULT MAN. $\times 90$. On the right the body, P' , and the dendrites, P'' , of a cell of Purkinje are sketched to demonstrate the difference between this element and the glia-cells. Techn. No. 74.

The *pineal body* (epiphysis, conarium,) is derived from a diverticulum of the primitive brain-vesicle and consists of epithelial cells, some of which have delicate processes, and of a connective-tissue envelope from which septa extend into the interior of the body, in which almost invariably the so-called "brain-sand" is found, rounded concretions varying in size and having uneven, mulberry-like surfaces (Fig. 99). They are composed of an organic basis and calcium phosphate with magnesium phosphate.

Not infrequently (especially in advanced life) there occur in the brain substance round or discoid bodies exhibiting distinct concentric striation, staining violet on treatment with iodine and sulphuric acid, and therefore related to amyllum (Fig. 100, *a*). These *corpora amylacea* are almost constant within the walls of the ventricles of the brain, and are also present in many other localities, as well in the gray as in the white substance.

FIG. 98.—PORTION OF HORIZONTAL SECTION OF HUMAN PITUITARY BODY, showing the boundary line between the anterior and posterior lobe. Two gland-tubules on the left contain each a dark epithelial cell. $\times 220$. Techn. No. 75.

THE MEMBRANES OF THE CENTRAL NERVOUS SYSTEM.

Two connective-tissue membranes envelop the brain and the spinal cord: the *dura* and the *pia*.

The *dura* of the *spinal cord* consists of compact connective tissue and numerous elastic fibers, flat connective-tissue cells and plasma-cells (Fig. 103). The inner surface is covered by a simple layer of flat epithelial cells (endothelium). The nerves and the blood-vessels are not numerous.

The *dura* of the *brain* forms also the periosteum of the inner surface of the cranium and consists of two lamellæ; an inner, corresponding to the *dura* of the cord, and of like structure, and an outer, corresponding to the periosteum of the vertebral canal. It is composed of the same elements as the inner lamella, with the exception that the outer fiber-bundles are disposed transversely to the inner. The outer lamella is rich in blood-vessels, which pass from it into the cranial bones.

The *pia* of the brain and spinal cord is a two-layered sack. The outer layer, the *arachnoid* of authors, is covered on its free surface by a simple layer of epithelium (endothelium), and is not closely attached to the *dura*. The inner layer (*pia*) closely invests the surface of the brain and cord, and sends

FIG. 99.—ACERVULUS CEREBRI FROM THE PINEAL BODY OF A WOMAN SEVENTY YEARS OLD. $\times 50$. Techn. No. 76.

FIG. 100.—FROM A TEASED PREPARATION OF GRAY SUBSTANCE FROM THE WALL OF A VENTRICLE OF HUMAN BRAIN. $\times 240$. *a*, Corpora amylacea; *b*, myelin drops; *c*, red blood-corpuscles; *d*, ependymal cells; *e*, medullated nerve-fibers; *f*, ganglion-cell. Techn. No. 77.

into their substance processes carrying blood-vessels. The *arachnoid* and the *pia* are joined together by numerous trabeculæ extending from the inner surface of the former to the outer surface of the latter. Hernia-like evaginations occur on the outer surface of the *arachnoid* in certain localities, in particular near the superior longitudinal sinus, and push the attenuated *dura* before them into the venous sinus. These are the so-called *villi of the arachnoid*, which under the name *Pachionian bodies* were long regarded as pathologic. The *pia* is composed of delicate connective-tissue bundles and plate-like cells which cover the inner surface of the *arachnoid* and the trabeculæ.

The *telæ choroideæ* and *plexus choroideæ* are highly vascular villous processes on the margin of a fold of the *pia* that hang like a fringe within the ventricles; they consist of connective tissue and blood-vessels, whose fine ramifications are united into tufts or lobules. They are covered by a simple layer of cubical epithelial cells, ciliated in the newborn, which enclose pigment-granules or oil-globules.

THE VESSELS OF THE CENTRAL NERVOUS SYSTEM.

The *blood-vessels* of the central nervous system form a narrow-meshed capillary network in the gray, a wide-meshed network in the white substance. The blood-vessels possess a so-called adventitial sheath (perivascular lymph-sheaths) often consisting of only a simple stratum of endothelial cells. The walls of the intradural venous sinuses are composed of a simple endothelial membrane.

The *Lymph Channels*.—Between the dura and the arachnoid there is a capillary cleft or fissure, the *subdural space*, which communicates with the deep cervical lymph-vessels and lymph-nodes (at least in the rabbit and the dog), with the lymph channels of the peripheral nerves, with the lymph-vessels of the nasal mucous membrane, with the smaller clefts (juice-canals) in the dura, and finally, round the arachnoidal villi, with the intradural venous sinuses. The fluid in the subdural space is very scanty.

The *subarachnoidal space*, that between the two layers of the pia—(arachnoid and pia)—communicates with the “juice channels” of the peripheral nerves, the lymph-vessels of the nasal mucous membrane, the interior of the ventricles of the brain and of the central canal of the spinal cord. The fluid in the subarachnoid space is very abundant; it is called the *cerebro-spinal fluid*.

The perivascular lymph-spaces are also in communication with the subarachnoid spaces, and may be injected from the latter.

The spaces filled only by injecting the brain substance itself cannot be included in the system of lymphatic channels. These spaces occur as *pericellular spaces* surrounding the larger ganglion-cells of the cerebral cortex, also many glia-cells; as *perivascular spaces* of the blood-vessels, that formed by the adventitial sheath excepted; and between the pia and the cerebrum, as the *epicerebral space*. These may be regarded as a separate juice-canal system.

2. THE PERIPHERAL NERVOUS SYSTEM.

THE NERVE-TRUNKS.

The *cerebro-spinal nerve-trunks* are composed chiefly of medullated nerve-fibers varying in thickness and only a few gray nerve-fibers, and therefore by reflected light appear white. Their mode of union agrees in many respects with that of the striated muscle-fibers. A sheath formed of loose connective tissue and elastic fibers, often containing clusters of fat-cells, surrounds the entire nerve-trunk. It is called the *epineurium* (Fig. 101). Extensions of the epineurium into the interior of the nerve surround the so-called secondary nerve-fiber bundles (funiculi), each of which is enveloped by the concentrically lamellated connective-tissue *perineurium*. From the latter connective-tissue septa extend into the interior of the secondary nerve-fiber bundles; they

constitute the *endoneurium*. Finally, delicate offsets from the endoneurium, the *fibrillar septa*, corresponding to the endomysium of the single muscle-fiber, surround each individual nerve-fiber. These sheaths are in direct connection with the tissue of the dura and the pia. Perineurium and endoneurium are composed of bundles of fibro-elastic tissue arranged in a number of lamellæ

FIG. 101.—PORTION OF CROSS-SECTION OF HUMAN MEDIAN NERVE. $\times 20$. Techn. No. 79.

concentrically disposed; each lamella is lined by a simple layer of flattened connective-tissue cells, whose outlines can be demonstrated by silver staining. The fibrillar septa also consist of delicate connective-tissue bundles lined by endothelioid plates.

FIG. 102.—PORTION OF CROSS-SECTION OF THE HUMAN MEDIAN NERVE. $\times 220$. Techn. No. 79.

The nerve-fiber bundle not infrequently divides; a variable number of nerve-fibers branch off from one funiculus to join another, and the result is a plexus of nerve-fiber bundles. Division of the nerve-fibers does not occur until at the periphery.

The *sympathetic nerve-trunks* are in part white and in part gray in color, depending upon the greater or lesser number of medullated nerve-fibers present :

for example, the splanchnic nerves contain many medullated nerve-fibers, while the gray branches of the abdominal and pelvic plexuses contain only a few of the thinnest medullated and, on the other hand, numerous nonmedullated nerve-fibers. The nerve-fibers are held together and grouped into bundles by connective tissue.

The blood-vessels run lengthwise within the epineurium, and form within the perineurium and endoneurium capillary networks with elongated meshes.

The lymph channels are represented by the spaces between the lamellæ of the perineurium and between the individual nerve-fibers; these are in communication with the sub-dural and the sub-arachnoidal spaces, but not with the lymph-vessels accompanying the nerve-trunk.

FIG. 103.—PIECE OF CROSS-SECTION OF THE GASSERIAN GANGLION OF MAN. $\times 240$. The cell-processes cannot be seen in such sections as this. At X the protoplasm of the ganglion-cell has retracted and simulates a process. In the axis of the transversely cut nerve-fibers the axis-cylinders are seen in section. Techn. No. 80.

THE GANGLIA.

Ganglia are groups of nerve-cells occurring along the course of the nerve-trunks. They are usually macroscopic in size, and contain small bundles of nerve-fibers between which lie ganglion-cells arranged in rounded groups or in longitudinal rows. A connective-tissue capsule, an extension of the epineurium, covers the outer surface of the ganglion and sends into the interior delicate processes for the support of the nerve-fibers and ganglion-cells. The blood-vessels are very numerous and form a capillary network which surrounds the individual cells.

The cells of the spinal ganglia are bipolar in the embryo; the processes spring from opposite poles of the cell. In the course of development the two processes gradually approach until finally they proceed from a common stalk; the cell thus becomes unipolar. (In amphibians and birds

isolated multipolar ganglion-cells occur; their dendrites are, however, short and only slightly branched.) The process of the adult cell receives a medullary sheath and a neurilemma very near its exit, and after a short course divides at a node of Ranvier into two T- or Y-branches. One branch—the cellulipetal—passes as the axis-cylinder of a sensory nerve-fiber to the periphery of the body, the other—the cellulifugal—usually the thinner, enters the spinal cord as a constituent of a posterior nerve-root, and terminates in free branches in the gray substance. Thus each spinal ganglion-cell, by its undivided process, is in a manner intercalated in the course of a sensory nerve-fiber. The cells of the spinal ganglia are large, round, often pigmented, and their vesicular nucleus contains a conspicuous nucleolus. Each ganglion-cell is enveloped in a nucleated capsule consisting of concentric strata of flat connective-tissue cells, which is prolonged on to the process of the cell as the *fiber-sheath* (neurilemma).

Whether any nerve-fibers pass through the spinal ganglia that do not enter into relation with the ganglion-cells is uncertain. In young embryo chicks such fibers have been seen coming from the cells of the anterior cornua, but they have not been found in mammals.

FIG. 104.—PORTION OF A SECTION OF THE SUPERIOR CERVICAL GANGLION OF MAN. $\times 240$. Techn. No. 81.

Gray nerve-fibers from the sympathetic occur in the spinal ganglia; they branch and form a plexus in the connective-tissue capsule of the ganglion-cells.

Other ganglia possessing the same structure as the spinal ganglia are: the Gasserian, the jugular, the plexus nodosus of the vagus, the petrosal, and the geniculate; the ganglion of the auditory nerve (ganglia nervi cochleæ et nervi vestibuli) contains bipolar cells.

The *sympathetic ganglia* consist of nerve-fibers and small, often pigmented, cells surrounded by a nucleated capsule and possessing one or two nuclei (two in the rabbit and the guinea-pig). The cells are multipolar; the axis-cylinder process passes directly into a nerve-fiber; the varicose ramifications of the protoplasmic processes surround the neighboring ganglion-cells. The nerve-fibers are partly thin medullated, partly nonmedullated fibers; their terminal ramifications in part surround the ganglion-cells. The nerve-cells of the sympathetic ganglia of fishes are bipolar. In amphibians ganglion-cells occur in which the single process with T-branches is surrounded by a spiral fiber.

THE PERIPHERAL NERVE-ENDINGS.

TERMINATIONS OF SENSORY NERVES.

The peripheral terminal branches of the sensory nerves are distributed naked, as *free endings*, or they are enclosed by epithelial or connective-tissue cells and terminate as *special endings* (terminal corpuscles, end-organs).

Free endings are formed as follows: a medullated nerve-fiber in passing to its ultimate distribution loses its medullated sheath, divides repeatedly, and forms a plexus of primitive fibrils which terminate in pointed or club-shaped ends. These endings occur more particularly in stratified epithelium. They

FIG. 105.—PERPENDICULAR SECTION THROUGH THE SKIN OF THE GREAT TOE OF A MAN TWENTY-FIVE YEARS OF AGE. $\times 200$. The cell-nuclei of the stratum mucosum are distinct only in the deepest layer. *L*, Cells of Langerhans; *n*, intraepithelial nerve-fibers. *P*, *P*¹, Two papillae of the corium: *P*, contains a capillary loop, *e*, of which only one limb is visible: *P*¹, contains a tactile corpuscle, *t*, with two approaching medullated nerve-fibers. Both papillae contain nonmedullated nerve-fibers. Techn. No. 82.

have been demonstrated in the cornea, in the oral mucous membrane, and in the deeper strata of the epidermis. In the latter cells with long branched processes—the *cells of Langerhans*—occur; these were formerly regarded as migrated wandering cells from the corium, and it is possible that some of them may have such an origin; the majority, however, are transformed epithelial cells; all the transitional forms from the typical epithelial cells to the stellate bodies in question may be found.

Sensory nerves have also been found in the muscles. The nerve-fibers lose their medullated sheath and, invested only by the nerve-nuclei, divide dichotomously; the delicate naked fibrillae extend lengthwise between the muscle-fibers and terminate in free endings.

The *terminal corpuscles* or *special endings* may be divided into two groups: *tactile-cells* and *end-bulbs*. In the tactile-cells the nerve-fiber terminates in relation with one or two cells; in end-bulbs it terminates in the interior of a finely granular body, the so-called inner bulb.

FIG. 106.—FROM A PERPENDICULAR SECTION THROUGH THE SKIN OF THE GREAT TOE OF A MAN TWENTY-FIVE YEARS OLD. $\times 240$. The outlines of the cells and nuclei of the epidermis can only be seen indistinctly. x. Tactile-cells in the corium, resting upon the ramifications of a delicate nerve-fiber. Techn. No. 82.

TACTILE-CELLS.

These may be either *simple* or *compound*. The *simple tactile-cells* are oval nucleated bodies measuring 6 to 12 μ (Fig. 106); they occur in the deeper strata of the epidermis or adjacent portion of the corium, and are embraced by the *tactile meniscus*, a crescentic expansion in relation with a nonmedullated nerve-fiber.

The *compound tactile-cells* (Grandry's and Merkel's) consist of two or more somewhat flattened cells, each larger than a simple tactile-cell (15 μ deep

FIG. 107.—FROM PERPENDICULAR SECTIONS THROUGH THE SKIN OF THE BEAK OF A GOOSE. $\times 240$. A. Compound tactile-cell (simple tactile corpuscle), cut parallel to the course of the entering nerve-fiber: n, medullated nerve fiber only partially met by the section; a, axis-cylinder; its division is here, in profile, invisible; ts, tactile disks cut perpendicularly; c, connective-tissue sheath; ts, tactile-cells. B. Two compound tactile-cells cut transversely to the plane of the entering nerve-fiber: 1, "Simple tactile corpuscle" consisting of four tactile cells; 2, twin tactile-cells; ts, tactile disks; a, axis-cylinders in transverse section, before dividing; n, medullated nerve fibers; c, corium. Techn. No. 83.

by 50 μ wide) and containing a vesicular nucleus. Between the cells is a flattened *tactile disk*, which is embraced between the forks of the divided axis-cylinder of a medullated nerve-fiber. The medullary sheath terminates at the point where the fiber enters the corpuscle and the neurilemma becomes fused with the connective tissue of the capsule surrounding the tactile-cells (Fig. 107). The compound forms containing three or four tactile-cells have been

designated "simple tactile corpuscles." The compound tactile corpuscles have only been found in the epidermis of the beak and in the tongue of birds, especially in web-footed birds; they are situated almost exclusively in the uppermost strata of the corium.

END-BULBS.

The end-bulbs are spheroidal or oval bodies in the interior of which a nerve-fiber terminates in a simple or branched ending. There are various forms of end-bulbs.

The so-called *cylindrical end-bulbs*, the simplest form, consist chiefly of a modified extension of the entering nerve-fiber and comprise three parts, the *axis-cylinder*, the *inner bulb*, and the *capsule*. The capsule is a continuation of the connective-tissue sheath of the nerve-fiber. The inner bulb is a

FIG. 108.—CYLINDRICAL END-BULB FROM THE CONJUNCTIVA OF CALF. $\times 240$. Techn. No. 84.

FIG. 109.—SMALL CORPUSCLE OF VATER FROM THE MESENTERY OF CAT. $\times 50$. The cells lining the capsules may be recognized by their prominent nuclei. The medulla of the nerve-fiber may be traced to the inner bulb. Techn. No. 85.

finely granular mass exhibiting concentric striations and a few nuclei at the periphery. The nerve-fiber loses its medullary sheath before entering the inner bulb, into which the axis-cylinder ascends as a flat band and terminates at the upper pole in a free or club-shaped ending. The cylindrical end-bulbs are found in the tunica propria of mucous membranes; for example, in the scleral conjunctiva and the oral mucous membrane.

The *corpuscles of Vater*, or *Pacinian bodies*, are transparent, elliptical forms, 2 to 3 mm. long and 1 to 2 mm. thick, and like the cylindrical end-bulbs consist of a capsule, an inner bulb, and an axis-cylinder; the two latter possess the same structure as in the end-bulbs, but the capsule is differently formed. It consists of twenty-five to fifty concentric lamellæ, each lined by a simple layer of endothelioid cells and separated from neighboring lamellæ by a serous fluid. Each lamella consists of an outer transverse and an inner longitudi-

nal layer of connective-tissue fibers. They are thinner and closer together near the inner bulb. Along the course by which the entering nerve passes to the inner bulb the lamellæ are not infrequently united by a longitudinal strand of tissue, the *interlamellar* ligament. A small artery accompanies the nerve-fiber into the interior of the corpuscle and breaks up into a capillary network between the concentric lamellæ.

The Pacinian bodies or corpuscles of Vater are found in the subcutaneous connective tissue of the palm of the hand and the sole of the foot, on the pudic nerves of the penis and the clitoris, in the vicinity of joints, in the neighborhood of the pancreas, in the mesentery, and elsewhere.

Not infrequently the axis-cylinder terminates in a forked ending or in a number of twisted and interlacing branches.

The corpuscles of Herbst and Key-Retzius, occurring in birds, closely resemble the corpuscles of Vater; they differ only in being much smaller and in possessing a double row of longitudinally-disposed nuclei in the inner bulb.

The *genital corpuscles* of the lower mammals and of man are spherical or oval forms (0.06 mm. wide by 0.4 mm. long), and consist of a finely granular nonnucleated inner bulb enveloped in a connective-tissue capsule containing cells rich in protoplasm. The approaching medullated nerve-fibers make several turns around the corpuscle, lose their medulla, and divide; the naked axis-cylinders penetrate the inner bulb at different points, undergo rapid division, and form a dense plexus of fibrils with varicose enlargements. In imperfect staining the varicosities simulate club-shaped endings. Each plexus is joined to neighboring plexuses by delicate nervous filaments.

The genital corpuscles lie in the depths of the corium at various distances from the papillary stratum; in the papillæ only smaller corpuscles, resembling the "simple spherical end-bulbs," are found. The largest number, one to four to the square mm., occurs in the glans penis and the clitoris. The so-called *simple spherical end-bulbs* (they are sometimes oval) have a similar structure; they are found in the conjunctiva and the adjoining portions of the cornea, and possess a greatest diameter of 0.02 to 0.1 mm. The *articular corpuscles* belong to the same category.

The *tactile corpuscles* (Wagner's and Meissner's) are elliptical structures, 40 to 100 μ long and 30 to 60 μ broad, characterized by cross markings. They possess a connective-tissue capsule with flattened cells, the boundaries of which and their transversely placed nuclei produce the striations. Two or more medullated nerve-fibers enter each corpuscle; after a number of winding excursions about the inferior pole of the corpuscle, their connective-tissue sheath blends with the tissue of the capsule, they lose their medullary sheath, and enter as

FIG. 110.—TACTILE CORPUSCLE FROM A PERPENDICULAR SECTION OF THE GREAT TOE OF A MAN TWENTY-FIVE YEARS OLD. $\times 560$. *a*, Medullated nerve-fibers; *e*, varicosities; *h*, connective-tissue sheath. The nuclei are not visible. Techn. No. 82.

naked axis-cylinders into a granular substance corresponding to an inner bulb; there they form a complicated plexus beset with varicosities. These tactile corpuscles lie in the papillæ of the corium and are most numerous (23 to one square mm.) in the skin of the palm of the hand, the finger-tips, and the plantar surface of the foot.

FIG. 111.—MOTOR NERVE-ENDING OF INTERCOSTAL MUSCLE-FIBERS OF RABBIT. $\times 150$. Techn. No. 86 a.

TERMINATIONS OF THE MOTOR NERVES.

The medullated nerve-fibers supplying striated muscle divide into branches, and these subdivide into twigs (nerve-fiber bundles) which anastomose and form a plexus, the *intramuscular plexus*. In the vicinity of this plexus the medullated nerve-fibers undergo numerous divisions, so

FIG. 112.—MOTOR NERVE-ENDING IN A FIBER OF AN OCULAR MUSCLE OF RABBIT. $\times 240$. *N*, Medullated nerve-fiber; *K*, nuclei of the disk. The transverse striæ are distinct only in the lower half of the muscle-fiber. Techn. No. 86 b.

that the number of nerve-fibers is considerably increased. From the small bundles of the plexus single delicate nerve-fibers spring, each one of which finally unites with a muscle-fiber. At the point where the nerve-fiber comes into contact with the muscle-fiber it loses its medullated sheath, the axis-cylinder breaks up into a number of slightly tortuous terminal branches with bulbous, swollen extremities, which form the so-called *motor end-plate*, which rests upon a rounded, finely-granular disk-like *sole-plate* containing numerous vesicular nuclei. Each muscle-fiber possesses at least one motor end-plate; whether these

lie upon or under the sarcolemma is not yet definitely determined.

The nonmedullated nerves supplying the smooth muscles form a *ground plexus* consisting of small bundles of nerve-fibers; branches of this plexus divide repeatedly and form networks, the *intermediate plexus*, from which small

bundles of fibrillæ arise and extend to the muscle-fibers; they are often slightly thickened at the point of contact with the muscle-fiber.

3. THE SUPRARENAL BODY.

The description of the suprarenal body with the organs of the nervous system is warranted by the profusion of its nervous elements, by its relation to the central nervous system as established by experiment, as well as by the facts of comparative anatomy.

Each suprarenal body consists of a cellular parenchyma and a connective-tissue capsule, which sends delicate processes into the interior of the organ.

FIG. 113.—A. SECTION OF THE SUPRARENAL BODY OF A CHILD. $\times 15$. Techn. No. 87. B. SECTION OF HUMAN SUPRARENAL BODY. $\times 50$. Techn. No. 89.

The parenchyma consists of an outer stratum, the *cortex*, which surrounds an inner mass, the *medulla*, on all sides. The cortex in the fresh state is of a yellow color and is composed of groups of cells about $15\ \mu$ in size, rounded in shape, possessing a coarsely-granular protoplasm, sometimes containing fat particles, and a clear nucleus. According to the arrangement of these cells, three zones are distinguished: 1, the *zona glomerulosa*; 2, the *zona fasciculata*; 3, the *zona reticularis*. The medulla in the fresh state is sometimes lighter, sometimes darker than the cortex; it consists of polygonal cells possessing a finely-granular protoplasm and a clear nucleus, which are arranged in oval groups or cords joined in an irregular network.

In the outer zone—*zona glomerulosa*—the cells are grouped in oval masses; in the middle zone—*zona fasciculata*—they are arranged in cylindrical columns, and in the innermost zone they are irregularly scattered in anastomosing cords. This stratum is distinguished by its pigment-cells.

The arteries divide in the capsule into numerous branches, which penetrate the cortex and there form a long-meshed capillary network, which passes into the medullary substance where the meshes are round. From the latter the veins proceed, of which the larger are accompanied by longitudinally-disposed bundles of smooth muscle-fibers. While still within the medulla the veins unite and form the chief vein, the suprarenal.

The numerous nerves (in man about 33 small stems) enter the cortex with the arteries and pass to the medullary substance, where they form a close reticulum. They are nonmedullated fibers, chiefly from the coeliac plexus, accompanied by groups of ganglion-cells, that are found even in the medulla.

V. THE DIGESTIVE ORGANS.

MUCOUS MEMBRANES.

The inner surface of the alimentary tract, of the respiratory passages, parts of the genito-urinary system, and parts of the organs of special sense are covered by a soft, moist membrane, the *mucous membrane*, or *tunica mucosa*. It is composed of a soft epithelium and of connective tissue. Immediately under the epithelium, the latter is usually specialized and condensed to form a structureless membrane, the *membrana propria* or *basement membrane*; beneath this follows the tunica propria, which passes by a gradual transition into the subjacent, loose-textured *tunica submucosa*, which in turn connects the mucous membrane with the underlying structures, the muscles or bones. The epithelium of the glands is derived directly from the epithelial elements covering the mucous membrane.

THE MUCOUS MEMBRANE OF THE ORAL CAVITY.

The mucous membrane of the mouth consists of two parts: (1) the epithelium and (2) the tunica propria; beneath the latter is the submucosa. The *epithelium* is typical stratified squamous epithelium. The *tunica propria* is formed of interlacing connective-tissue bundles richly interspersed with elastic fibers. The bundles of the uppermost strata are very slender and form a compact, apparently almost homogeneous feltwork. The surface of the tunica propria is beset with numerous usually simple papillæ, varying greatly in height (Fig. 114). The highest papillæ (0.5 mm.) occur at the edge of the lips and on the gums. The tunica propria passes without sharp limits into the *submucosa*, which consists of somewhat thicker bundles of connective tissue, among which

the elastic fibers are not numerous. The submucosa is in general loosely attached to the walls of the oral cavity; only on the gums and the hard palate is it firmer, and intimately united to the periosteum. It contains the glands of the mucous membrane; these are, with the exception of the sebaceous glands occasionally found at the edges of the lips, branched tubular mucous glands from 1 to 5 mm. in size. The main excretory duct is somewhat expanded at its lower end and in the greater part of its extent is lined with stratified scaly epithelium; the branches and twigs into which it divides and subdivides are lined with stratified squamous and simple columnar epithelium respectively. Not infrequently the main excretory duct receives the excretory tubes of small accessory glands

FIG. 114.—VERTICAL SECTION THROUGH THE MUCOUS MEMBRANE OF LIP OF ADULT MAN. X 30. 1. Papilla; 2, excretory duct; the lumen is cut open at one point only; 3, accessory gland; 4, a branch of the excretory duct in transverse section; 5, gland-follicles grouped into lobules by connective tissue; 6, a gland-tubule in transverse section. Techn. No. 91.

(Fig. 114, 2, 3). The minute structure of the gland-tubules will be described with the glands of the tongue. The numerous blood-vessels of the oral mucous membrane are arranged in two networks, situated in two planes, of which the coarser lies in the submucosa, the capillary network in the tunica propria. From the latter terminal capillary loops ascend to supply the apices of the papillæ. The *lymph-vessels* also form two networks, a coarser in the submucosa, a finer in the tunica propria. The medullated nerve-fibers form a wide-meshed reticulum in the submucosa, from which many ramifying fibers ascend to the tunica propria where they terminate in end bulbs, or lose their medullary sheath and as nonmedullated nerve-fibers penetrate the epithelium, where after repeated division they terminate in free endings between the epithelial cells.

THE TEETH.

The teeth of man and the higher animals are solid structures, possessing a central cavity—the *pulp-cavity*—filled with a soft mass, the *pulp*. The portion of the tooth within the alveolus or socket is called the *fang*, the exposed portion, the *crown*; the juncture of these portions forms the *neck*; the latter is covered by the gums. The hard substance of the tooth consists of three different parts: (1) the *dentine*, (2) the *enamel* with the *enamel cuticle*, and

FIG. 115.—LONGITUDINAL SECTION OF HUMAN TOOTH. $\times 4$. Techn. No. 92.

(3) the *cementum*. The distribution of the parts is as follows: the dentine contributes the chief bulk of the tooth and determines its form; it completely encloses the pulp-cavity except where a small nutrient canal at the apex of the fang admits the numerous blood-vessels to the pulp; the dentine of the crown is covered by the enamel, of the fang by the cementum, so that its surface is nowhere exposed (Fig. 115).

The *dentine* or *ivory* is a white, opaque mass, harder than bone. It consists of an apparently homogeneous ground-substance composed of extremely

fine fibrillæ, and is pierced by numerous minute channels, the *dentinal tubules*. The latter begin with a diameter of about 2.5μ at the inner surface of the dentine, describe an S-shaped curve, and then proceed in a slightly wavy course, radially directed toward the outer surface, steadily decrease in caliber, and terminate at the juncture of the dentine and enamel or cementum, or they form a

FIG. 116.—FROM A LONGITUDINAL SECTION OF THE LATERAL PART OF THE CROWN OF A HUMAN MOLAR TOOTH. $\times 240$. 1. Dentinal tubules, extending for a short distance into the enamel; 2, dentinal globules projecting into, 3, the interglobular spaces. Techn. No. 92

loop and turn into a neighboring tubule. During their course they send off numerous lateral branches which establish communication with surrounding canaliculi. The matrix immediately surrounding the dentinal tubules is especially dense, and forms the so-called *dentinal sheaths*. The lumen of the *dentinal tubules* is occupied by the *dentinal fibers*. At the periphery of the dentine are the *inter-*

FIG. 117.—FROM A LONGITUDINAL SECTION OF THE FANG OF A HUMAN MOLAR TOOTH $\times 240$. 1. Dentinal tubules interrupted by a granular stratum, with many, 2, small interglobular spaces; 3, bone corpuscles with many processes. Techn. No. 92.

FIG. 118.—FROM AN INFANT. Techn. No. 93.

globular spaces, irregular clefts varying in size and filled with a soft mass; into these spaces the dentine juts in the form of hemispherical protuberances, the *dentinal globules*. At the neck and in the fang are many very small interglobular spaces, which form the so-called granule stratum lying immediately beneath the cementum.

The *enamel* is still harder than the dentine. It is composed exclusively of long hexagonal homogeneous columns, 3 to 6 μ in thickness—the *enamel prisms*—which are firmly united with one another by a scanty amount of aqueous cement-substance. They extend radially, with many undulations, from the surface of the dentine to the outer surface of the enamel; the latter is covered by a very thin but very resistant membrane, the *enamel cuticle* or membrane of Nasmyth.

The *cementum* (*crusta petrosa*) coincides in structure with bone. It contains many Sharpey's fibers. Haversian canals are found only in the cementum of older individuals; stratification in the lamellæ is seldom well-defined. Bone-corpuscles are absent near the neck.

The space between the fang and the alveolus is occupied by the richly-innervated periosteum, which is firmly united to the cementum by Sharpey's fibers; they penetrate from the inferior maxilla through the periosteum into the cementum. The uppermost portion of the periosteum is called the *circular dental ligament*.

FIG. 119.—SIX ODONTOBLASTS WITH DENTINAL FIBERS.
f: ϕ . Pulp processes. From the pulp of an infant. $\times$
240. Techn. No. 94

The *pulp* is formed of a soft connective tissue containing delicate fibers, not united into bundles, and whose cellular elements, at the surface in contact with the dentine, form a layer of elongated nucleated cells, the *odontoblasts*; these send out short processes, by which they connect with other elements in the pulp, and long processes that

extend into the dentinal tubules as the above-mentioned *dentinal fibers* (Fig. 119f). Blood-vessels and nerves are limited to the pulp.

DEVELOPMENT OF THE TEETH.

The development of the teeth in man begins toward the close of the second month of fetal life,* and is first indicated by a linear proliferation of the primitive epithelium, which in the form of a continuous projection grows obliquely into the subjacent connective tissue. This crest, the *dental ridge* ("enamel germ"), develops on its lateral (labial) surface knob-like protuberances, the *dental bulbs*, which correspond in number to the temporary teeth, and coincidentally in the surrounding mesoderm as many conical aggregations of closely-packed connective-tissue cells appear, the young *dental papilla* (tenth week). The latter advance obliquely from the labial to the lingual side and toward the surface, and

* That which, at an earlier period (fortieth day), has been described as the anlage, is not this alone, but includes the anlage of the labial furrow.

are embraced by the dental bulbs in such a manner that these form an epithelial cap for the dental papillæ. Thus each bulb becomes an *enamel organ*. At the same time the dental ridge has assumed a more nearly vertical position, and fol-

FIG. 120.—SCHEMATIC REPRESENTATION OF THE INITIAL PROCESSES IN THE DEVELOPMENT OF THE TOOTH, showing the formation of three teeth. The anlage of each anterior tooth is seen in section; the cut surface is stippled. *k*, Free edge of the dental ridge.

lowing the direction of the gums a longitudinal groove has appeared, the *dental furrow*, which indicates the line along which the dental ridge progressed backward toward the mandibular articulation. The time of the appearance of the dental furrow varies; it is frequently present in the initial stages. It disappears later.

FIG. 121.—FRONTAL SECTION OF THE HEAD OF AN EMBRYO SHEEP 4 CM. LONG. $\times 15$. Techn. No. 95.

The original broad attachment between the dental ridge and the enamel organ is diminished by partial constriction, and is finally reduced to a slender cord, the *isthmus*. Meanwhile, the papilla and enamel organ have developed beyond the

dental ridge, so that the free edge of the latter does not extend to half the depth of the enamel organ (Fig. 120 and Fig. 123). At the same time the elements of the enamel organ undergo differentiation. The inner layer of cells, resting upon the papilla, develop into tall columnar elements, the *inner enamel-cells*; their inner surface is provided with a cuticular border. The outer cells, on the other hand, steadily decrease in height, until finally they are reduced to thin plates, the *outer enamel-cells*; the cells between the inner and outer enamel-cells, by an abundant increase of the intercellular substance, become transformed into stellate anastomosing elements, and form the *enamel-pulp*. At the point where the inner enamel-cells bend over into the outer layer, the enamel organ grows down until it has reached the extremity of the anlage of the tooth, thus forming, in a measure, the mould or matrix in which the tooth develops. The determination of the shape of the future tooth is the first function of the enamel

FIG. 122.—CROSS-SECTION OF THE LOWER JAW OF A HUMAN EMBRYO FOUR MONTHS OLD. $\times 42$.
Techn. No. 95.

organ; the second, the production of the enamel, which only takes place in that portion of the inner layer covering the crown of the tooth. This portion may be named the *enamel membrane*. Each cell of this membrane deposits a substance which subsequently calcifies and becomes an enamel prism. The enamel-cells surrounding the fang take no part in the production of the enamel; they decrease in height and (as here the enamel-pulp soon disappears) apply themselves directly against the outer enamel-cells, the two layers forming the *epithelial sheath* of the fang (Fig. 124).

Before the production of enamel has begun, the first lamina of dentine has been formed (about the twentieth week). The superficial cells of the dental papillæ elongate and become the *odontoblasts*, the agents which produce the at first uncalcified dentine. These cells do not develop beyond the extent of the epithelial sheath. As soon as the first dentine is formed, the epithelial

sheath undergoes retrogressive change; connective-tissue ingrowths from the alveolar periosteum penetrate between the epithelial cells. This retrogression begins at the lower border of the enamel organ, thus severing the connection between the latter and the deeper parts of the epithelial sheath. With the completed growth of the tooth the last remnant of the epithelial sheath disappears.

Even before the production of enamel and dentine the connection between the dental ridge and the oral epithelium is severed, and the mesodermic tissue

FIG. 123.—FROM A CROSS-SECTION OF THE UPPER JAW OF A HUMAN EMBRYO FIVE MONTHS OLD. $\times 42$.
Techn. No. 95.

surrounding the anlage of the tooth forms a compact membrane, the *dental sack*, in which subsequently an inner looser, and an outer denser, stratum can be distinguished.* The enamel cuticle and the cementum do not appear until after birth, shortly before the irruption of the teeth. The former is produced by the merging of the cuticular borders of the enamel-cells into a firm homogeneous membrane; the latter is a product of the alveolar periosteum.

*The dental ridge has previously become a perforated plate, beset on all sides with short, jagged excrescences. Remains of the dental ridge may be found in the gums of newborn children, and were formerly erroneously regarded as glands (*glandule tartaricæ*).

The permanent teeth develop in the same manner as the temporary teeth ; in the twenty-fourth week new protuberances develop on the growing ledge of the dental ridge, which embrace new papillæ arising from the sides. The anlage of the permanent tooth lies at first in the same alveolus with the temporary

FIG. 124.—LONGITUDINAL SECTION OF A YOUNG MILK-TOOTH OF A NEW-BORN DOG. $\times 42$. Techn. No. 95.

tooth ; a separate alveolus is developed later. The completed tooth is in part of epithelial origin (the enamel), and in part derived from the connective-tissue dental papilla (the dentine), the remains of which persist in the adult as the pulp. The cementum is in a measure an accessory formation contributed by neighboring tissues.

THE TONGUE.

The bulk of the tongue is formed of striated muscles, the separate bundles and fibers of which interlace in various directions. The unattached surfaces of the organ are covered by a reflection of the oral mucous membrane. The bundles of the muscular tissue are disposed in three planes: (1) *vertically*, and somewhat *radially* (geniohyoglossus, lingualis, and hyoglossus); (2) *transversely* (lingualis); and (3) *longitudinally* (lingualis and styloglossus). Since the muscle-bundles cross one another for the most part at right angles sections exhibit a regular, beautiful network. A median septum, the *septum linguae*, divides the muscular tissue into a right and a left half. The septum begins at the hyoid bone and gradually increases in height, reaching its greatest elevation in the middle of the tongue, then gradually slopes downward and

FIG. 125.—LONGITUDINAL SECTION OF THE MUCOUS MEMBRANE OF THE DORSUM OF THE HUMAN TONGUE. $\times 30$. 1. Section of two filiform papillae, each of which bears, 2, three secondary papillae; 3, compound, 4, simple process of epithelium, the surface of which is covered with masses of loosely-attached scaly epithelial cells.

FIG. 126.—LONGITUDINAL SECTION OF THE MUCOUS MEMBRANE OF HUMAN TONGUE. $\times 30$. 1. Fungiform papilla with, 2, secondary papillae; 3, stalk. 4. Small filiform papilla. Techn. No. 96.

forward and disappears; it does not extend through the entire half of the tongue, but ceases at a distance of about 3 mm. from the dorsum of the organ. The septum is composed of compact connective tissue.

The mucous membrane of the tongue, like that of the oral cavity, consists of an epithelium and a tunica propria, and rests on a submucosa. It is characterized by the conspicuous development and complicated form of the papillae. Three kinds of papillae are distinguished: the *filiform* or *conical*, the *fungiform*, and the *circumvallate papillae*.

The *filiform papillae* are cylindrical or conical elevations of the tunica propria, bearing on the summit five to twenty small secondary papillae. They are composed of distinctly fibrillated tissue and numerous elastic fibers, and are covered by a thick layer of stratified scaly epithelium that not infrequently, over the secondary papillae, forms a number of filamentous horny processes.

The filiform papillæ are very numerous, and are distributed over the entire dorsum of the tongue; they vary in height from 0.7 to 3 mm. (Fig. 125).

The *fungiform papillæ* are rounded elevations connected with the tunica propria by a slightly-constricted stalk; their entire surface is beset with secondary papillæ. They consist of a distinct feltwork of connective-tissue bundles which contain but few elastic fibers. The epithelial cover is thinner than that on the filiform papillæ and is not cornified. The fungiform papillæ are also distributed over the entire surface of the tongue, but are not so numerous as the filiform; they vary in height from 0.5 to 1.5 mm. They are usually easily distinguished by their red color, due to the capillaries shimmering through the transparent epithelium (Fig. 126).

The *circumvallate papillæ* resemble broad, flattened fungiform papillæ, and are separated from the surrounding epithelium by a circular furrow varying in depth and bounded by a ridge designated the *wall*. The papillæ are com-

FIG. 127.—SECTION OF A CIRCUMVALLATE PAPILLA OF MAN. $\times 30$. Techn. No. 96.

posed of connective tissue, like that of the fungiform papillæ. Secondary papillæ are found only on the upper surface. In the epithelium covering the sides, and occasionally also the wall, lie the end-organs of the special sense of taste—the *taste-buds*. The circumvallate papillæ are 1 to 1.5 mm. high and 1 to 3 mm. broad. They are eight to fifteen in number, and occur on the posterior end of the dorsum of the tongue. At the lateral margins of the tongue, just in front of the anterior pillars of the fauces, is a group of parallel folds of the mucous membrane—*papilla foliata*—containing numerous taste-buds. The papillæ foliatae are especially well developed in the rabbit.

The *submucosa* at the tip and at the edges of the tongue is firm and resistant and intimately connected with the underlying parts.

The Lymph-follicles of the Tongue.—The mucous membrane extending from the circumvallate papillæ to the epiglottis is peculiarly modified by the development of lymph-nodules. They are spherical aggregations of

adenoid tissue 1 to 4 mm. in size, embedded in the uppermost strata of the tunica propria, and form easily perceptible macroscopic elevations. In the center a punctate opening may be seen, the entrance to a deep central crypt.

FIG. 128.—VERTICAL SECTION OF A LYMPH-FOLLICLE FROM THE ROOT OF THE TONGUE OF ADULT MAN. $\times 20$. 1. Crypt of the follicle, containing migratory leucocytes. 2. Epithelium of the crypt; infiltrated with leucocytes on the left and at the base, almost intact on the right. 3. Nodules of adenoid tissue containing germinal centers; f^1 , nodules cut through the middle; f^2 , through the side; f^3 , at the periphery. 4. Fibrous sheath. 5. Section of excretory duct of mucous gland. 6. Blood-vessel. Techn. No. 96.

lined by a continuation of the stratified epithelium of the oral mucous membrane. The epithelium bordering the crypt is surrounded by diffuse adenoid tissue, which contains a variable number of the lymph-nodules, with germinal centers, and is separated by a sharp line of demarcation from the subjacent fibrillar connective tissue of the tunica propria; when well developed, the fibrous bundles of the tunica propria are circularly disposed about the adenoid tissue and form a fibrous capsule (Fig. 128, 4). Under normal conditions numerous leucocytes of the adenoid tissue wander through the epithelium into the central crypt and thence to the oral cavity; they are readily found in the saliva, as "mucous" and "salivary" corpuscles. The epithelium is often greatly expanded in consequence and destroyed, or is infiltrated with leucocytes to such a degree that its boundary cannot be definitely determined.

The Glands.—Two kinds of branched tubular glands occur in the mucous membrane and in the superficial muscular strata of the tongue. The gland-cells of the one kind produce a mucigenous secretion (mucin); such glands are named *mucous glands*. The secretion of the second kind is a thin, watery, serous fluid, distinguished by the large amount of albumin it contains; such glands are called *serous glands*.

FIG. 129.—FROM A SECTION THROUGH THE ROOT OF THE TONGUE OF MOUSE. $\times 90$. A serous gland; the duct-system silvered by Golgi's "black reaction"; the tubular structure is easily recognized. Tech. No. 119.

The *mucous glands* are of the same structure as those of the oral mucous membrane, and occur along the edges and—in larger numbers—at the root of the tongue, where not infrequently their excretory ducts open into the crypts of the follicles. The ducts are lined by columnar epithelium, which occasionally is ciliated; the walls of the tubules consist of a structureless *membrana propria* and gland-cells; the latter are columnar elements possessing a firm cell-membrane, and vary in appearance with their functional condition. The exhausted cell is smaller, the transverse-oval nucleus near the base of the cell; the cell loaded with secretion is broader, and the nucleus is pressed flat against the cell-wall. Generally the same gland, often the same tubule, exhibits varying phases of secretion; demilunes are however not formed here, because the rigid membrane of the gland-cells resists the pressure exerted by neighboring cells. Only the mucous glands of the tongue of the cat and of the uvula of man exhibit demilunes. Nuhn's glands occurring in the tip of the tongue are likewise mucous glands.

The *serous glands* are limited to the vicinity of the *papillæ circumvallatæ* and *foliatæ*; the excretory ducts open into the furrows between the *papillæ*

FIG. 130.—I, II. FROM A SECTION OF A MUCOUS GLAND OF THE ROOT OF HUMAN TONGUE. I. Section of a tubule with (b) gland-cells empty of secretion, and (c) gland-cells filled with secretion; d, lumen. II. Cross-section of a tubule containing only cells loaded with secretion. III and IV. From the mucous membrane of the tongue of rabbit. III. Tubule of a mucous gland in transverse section. IV. Several tubules of a serous gland, at d the very small lumen. V. Several tubules of a human serous gland, with large (d') and small (d) lumen. All the sections are magnified 240 times. Techn. No. 96.

and the wall, and are lined by simple or stratified columnar epithelium, not infrequently ciliated. The tubules consist of a delicate *membrana propria* and short cylindrical or conical cells, destitute of a membrane, whose dim granular protoplasm encloses a round nucleus. The lumen of the tubules is, especially in animals, very narrow.

The *blood-vessels* of the mucous membrane of the tongue form networks disposed parallel to the surface, from which twigs ascend to supply the *papillæ* and the secondary *papillæ*. At the root of the tongue small arteries pierce the fibrous envelopes of the lymph-follicles, and break up into capillaries that penetrate to the interior of the nodules. The blood-vessels of the glands form networks around the gland-tubules.

The *lymph-vessels* of the tongue are arranged in two sets; a deep set consisting of larger vessels, and a superficial set, which takes up the lymph-vessels of the *papillæ*. The lymph-vessels at the root of the tongue are very numerous; they form networks encircling the lymph-nodules.

The nerves of the mucous membrane of the tongue, the *glosso-pharyngeal*

and the lingual branch of the fifth, end in part as in other portions of the oral mucous membrane, and in part in intimate relation with the taste-buds.

THE PHARYNX.

The wall of the pharynx is composed of three coats: a *mucous*, a *muscular*, and a *fibrous coat*. The *mucous coat*, like the oral mucous membrane, possesses a stratified scaly epithelium, a tunica propria beset with papillæ, and also numerous mucous glands. The upper or respiratory part of the pharynx is clothed by stratified ciliated columnar epithelium; the lower limit of the latter is variable. Very richly developed is the adenoid tissue. Between the pillars of the fauces it forms conspicuous accumulations, one on either side, known as the tonsils, which in respect to their structure in man and many animals correspond to an aggregation of lymph-nodules like those of the root of the tongue. The leucocytes that wander through the epithelium of the tonsils are so numerous that the latter may be regarded as the most fertile source of the salivary corpuscles. The adenoid tissue is also vigorously developed in the respiratory portion of the pharynx, where on the posterior wall between the orifices of the Eustachian tubes it forms a conspicuous mass, the "pharyngeal tonsil," which agrees in its structure with the palatine tonsils, excepting that the lymphoid tissue is less sharply circumscribed. Here, too, many leucocytes migrate through the epithelium. The development of the adenoid tissue of the oral cavity and of the pharynx is subject to considerable variation.

The muscular coat (constrictor muscles of the pharynx) consists of striated muscle-fibers, the description of which belongs to the province of macroscopic anatomy. The *fibrous tunic* is a stout membrane composed of a dense feltwork of fibro-elastic tissue. Blood-vessels, lymph-vessels, and nerves are distributed in the same manner as in the oral mucous membrane.

THE ESOPHAGUS.

The walls of the esophagus comprise a mucous, a muscular, and a fibrous coat. The *mucous coat* is composed of a stratified squamous epithelium, of a tunica propria beset with papillæ, and following this of a stratum of longitudinally disposed smooth muscle-fibers, the *muscularis mucosæ*; subjacent to the latter is the *submucosa*, which consists of loosely-joined bundles of connective tissue, and in the upper half of the esophagus contains small mucous glands. The *muscular tunic*, in the upper portion of the tube, is composed of striated muscle-fibers, which in the lower portion are replaced by smooth muscle-fibers. The latter are arranged in two strata, an inner circular and an outer longitudinal layer. The fibrous coat consists of compact connective-tissue bundles interspersed with numerous elastic fibers. The distribution of the blood-vessels, lymph-vessels, and nerves is the same as in the pharynx. Between the circular and the longitudinal layers of the muscular coat the nerves form a plexus, at the nodal points of which minute groups of ganglion-cells occur (see Auerbach's plexus, p. 170).

THE STOMACH.

The wall of the stomach is 2 to 3 mm. thick and comprises four coats: a mucous, a submucous, a muscular, and a serous or fibrous tunic.

FIG. 131.—FROM A CROSS-SECTION OF THE MIDDLE THIRD OF HUMAN ESOPHAGUS. $\times 10$. 1. Stratified squamous epithelium. 2. Tunica propria. 3. Muscularis mucosæ. 4. Submucosa. 5. Circular muscles. 6. Longitudinal muscles. *g*. Blood-vessel. Techn. No. 98.

FIG. 132.—TRANSVERSE SECTION OF HUMAN STOMACH. $\times 15$. The tunica propria contains glands standing so close together that its tissue is visible only at the base of the glands toward the muscularis mucosæ. Techn. No. 99.

The *mucous coat*, sharply contrasted with the white esophageal mucous membrane by its reddish-gray color, consists of an epithelium, a tunica propria, and a muscularis mucosæ (Fig. 132).

The *epithelium* is a simple columnar epithelium, whose elements produce a mucoid secretion. Two zones can usually be distinguished, an upper mucoid

FIG. 133.—SECTION OF THE MUCOUS MEMBRANE OF THE CARDIAC END OF HUMAN STOMACH. $\times 220$
Techn. No. 102.

and a lower protoplasmic; the latter contains the oval, round, or flattened nucleus. The extent of the mucoid zone varies considerably with the func-

tional condition of the cells. After the discharge of their mucoid contents the epithelial elements closely resemble goblet-cells. The tunica propria is composed of a mixture of fibrillated and reticular connective tissue, and of an extremely variable number of leucocytes, that occasionally lie closely aggregated and form solitary lymphatic nodules. The tunica propria contains so many glands that its tissue is limited to delicate septa between, and to a thin stratum below, the tubules. In the pyloric end the glands are far apart, the tunica propria is conspicuously developed, and not infrequently elevated in filamentous or leaf-like villi.

Two kinds of gastric glands are recognized: *fundus glands*,* situated chiefly in the middle and cardiac thirds of the stomach, and *pyloric glands*, confined to the narrow pyloric region. Both kinds are simple tubular glands, often branched, especially in the pyloric region, and open singly or in groups into minute, pit-like *depressions* in the mucous membrane of the free surface. The portion of the gland adjoining these depressions is called the *neck*, the following portion the *body*, and the blind end the *fundus* (Fig. 133). Each gland consists of a membrana propria and of gland-cells.

The *fundus glands* contain two kinds of cells: *chief-* or *central-cells* and *parietal-* or *acid-cells*. The former are clear, cubical or short columnar cells,

FIG. 134.—TRANSVERSE SECTION OF A HUMAN FUNDUS GLAND. $\times 240$. Techn. No. 102.

whose granular protoplasm surrounds a spherical nucleus. The chief-cells are very unstable. The parietal-cells are marked by their affinity for anilin dyes, with which they react intensely. The two kinds of cells are not equally distributed; the chief-cells form the principal portion of the fundus, the parietal-cells are irregularly distributed, but are especially numerous in the neck and the body of the tubule. Here they lie in rows beside the chief-cells, but toward the fundus they are pressed to the periphery, without, however, being shut off from the lumen, with which they communicate by a short lateral canal extending between the chief-cells from the lumen to the parietal-cells. The lateral canal is the only one of the system of canaliculi enveloping the parietal-cells that can be seen in ordinary preparations. By the aid of Golgi's "black reaction," which also blackens the secretion, it may be seen that from the axial lumen of the fundus glands minute lateral twigs branch off at right angles, divide and anastomose, forming a fine-meshed network of "secretory capillaries" that, basket-like, embrace each parietal-cell. The secretion is discharged from all sides of the cell, passes into the secretory capillaries, then into one or

* In the earlier text-books the fundus glands were called peptic glands, a name based upon a function of the glands now called into question.

more short lateral canals, and finally into the lumen of the gland (Fig. 16 and Fig. 135).

The assertion upheld on various sides that the chief- and the parietal-cells are different functional appearances of one kind of cell, as also the statement that during digestion the parietal-cells multiply, but disappear after prolonged fasting, are very much in need of thorough investigation. The stomach of an animal killed after a long winter hibernation still contains parietal-cells.

The *pyloric glands* are furnished almost throughout with columnar cells containing a spherical nucleus situated near the base of the cell, which in the

FIG. 135.—CROSS-SECTION THROUGH THE MUCOUS MEMBRANE OF THE FUNDUS OF STOMACH OF MOUSE (DURING DIGESTION). $\times 234$. In the gland to the right the entire system of canaliculi, in the other glands only a portion of the same, is silvered. The "baskets" formed by the secretory capillaries can be distinguished. Techn. No. 119.

intermediate zone, that is, the border zone between the pyloric and the fundus mucous membrane, resemble very closely the chief-cells, with which they have been compared. In man isolated parietal-cells are found; in animals, *e. g.*, the dog, a few dark conical cells occur, that owe their appearance to the compression exerted by neighboring cells.

The foregoing description applies to the stomach as seen after a period of fasting; during digestion the parietal-cells are larger, the chief-cells, as also

the cells of the pyloric glands, are darker, the nuclei of the latter are nearer to the middle of the cell, and the secretory capillaries expanded with increased contents are wider than in the fasting organ.

The *muscularis mucosæ* consists of smooth muscle-fibers arranged in two or three layers interlacing in various directions, from which single strands branch off and ascend vertically between the gland-tubules (Fig. 133).

The *submucosa* is composed of loosely-united connective-tissue bundles and elastic fibers, and occasionally contains small clusters of fat-cells.

It is only in the pyloric region that two separate layers can be distinguished in the *muscular coat*, a thicker inner circular and a thinner outer longitudinal layer. In the other regions of the stomach the arrangement of the muscle-tissue is very complicated, owing to the extension of the muscular strata of the esophagus to the stomach, as well as by the curving of the organ that ensues in the course of development; sections exhibit bundles of fibers extending in every possible direction.

The *serous coat* will be described with the peritoneum.

THE INTESTINES.

The wall of the intestines, like the stomach, is composed of four tunics; a mucous, a submucous, a muscular, and a serous.

The *mucosa* is thrown into folds, the *valvæ conniventes*, especially well marked in the upper part of the small intestine, the object of which is to increase the superficial extent of the membrane. In addition to these readily perceptible plications there are still other contrivances serving a similar purpose,

FIG. 136.—LOWER PORTION OF A PYLORIC GLAND, FROM A PERPENDICULAR SECTION THROUGH THE MUCOUS MEMBRANE OF HUMAN STOMACH. $\times 240$. Techn. No. 102, b.

that stand at the limit of macroscopic perception. These are minute elevations and depressions of the mucous membrane. The former, the *villi*, are present only in the small intestine; in the large intestine of man they are wanting; they are processes about 1 mm. high, in the duodenum of leaf-like, in the remainder of the small intestine of cylindrical form. The depressions begin at the pylorus, and are found throughout the whole length of the intestine. They occur in their most primitive form in fishes, and originate in parallel folds running lengthwise that are connected by small transverse folds. In vertical sections these shallow depressions appear as short, wide sacks, called *crypts*. In mammals the crypts are deeper, their lumen narrower, and placed in rows close beside one another they have the appearance of simple tubular glands; but they could only be regarded as such if the epithelial cells lining them produced a specific secretion, which is not the case. Whether the isolated granular cells that occur in the fundus of the crypts are gland-cells is a question. The crypts are known as the *follicles* or *crypts* of *Lieberkühn*.

The mucous membrane consists of an epithelium, a tunica propria, and a *muscularis mucosæ*. The epithelium, which clothes the entire free surface,

including the villi, and lines the crypts is a simple columnar epithelium, the elements of which in their mature condition consist of a granular protoplasm containing numerous resorbed fat-particles, a usually oval nucleus, and a cell-membrane. On the free surface of the cells there is a homogeneous or finely-striated *basal border* characteristic of the intestinal epithelium.

The regeneration of the epithelium takes place only in the crypts of Lieberkühn, where by mitotic division new cells are constantly formed, which gradually move upward and replace the cells that disintegrate on the upper surface of the mucous membrane. Therefore the youngest generation of epi-

FIG. 137.—LONGITUDINAL SECTION OF THE JEJUNUM OF ADULT MAN. $\times 16$. The circular fold (valvula conniventes) on the right supports two small solitary nodules, that do not extend into the submucosa, and of which the left exhibits a germinal center, X . The epithelium is slightly loosened from the connective-tissue core of many of the villi, so that a clear space, XX , exists between the two. The isolated bodies lying near the villi (more numerous to the left of the valvulae conniventes) are partial sections of villi that were bent, and therefore not cut through their entire length. Techn. No. 105.

thelial cells is found in the crypts, the oldest on the free upper surface—in the small intestine on the apices of the villi. Goblet-cells in extremely variable numbers occur in the intestinal epithelium; they possess an elliptical or, not infrequently, a chalice form; the upper portion, that directed toward the surface of the intestine, undergoes different degrees of distention as the protoplasm is transformed into mucus, and the nucleus with the remainder of the unaltered protoplasm lies at the base of the cell; a basal border is wanting, in place of which a sharply-defined circular orifice is found, through which the mucus is poured out on the surface (Fig. 139, A).

The goblet-cells are derived from the ordinary epithelial cells of the intestine. In certain conditions each young intestinal epithelial cell may assume the functions of a goblet-cell.

FIG. 138.—SECTION OF THE MUCOUS MEMBRANE OF JEJUNUM OF ADULT MAN. $\times 80$. The empty space, *a*, between the tunica propria and the epithelium of the villi is an artificial product, the result of the shrinking action of the fixing fluid. Not infrequently within the space lie cells that have been pressed out of the tunica propria. In its retraction the epithelium often tears, and then the villus appears to have an opening, *b*, at its apex. The goblet-cells have been sketched on one side of the villus to the right. Techn. No. 105.

The several phases of secretion appear in regular sequence, and so that the later phases are always to be seen in the apices of the villi or near the upper surface of the mucous membrane, the initial phases in the crypts of Lieberkühn (Fig. 140).

FIG. 139.—INTESTINAL EPITHELIUM. $\times 560$. A. Isolated goblet-cells of rabbit. Techn. No. 104, *b*. X. Escaping mucus. B. From a section of the mucous membrane of human intestine. Techn. Nu. 102. *b*. A goblet-cell between columnar cells.

In the crypts of the small intestine the number of goblet-cells is proportionately less than in the large intestine; this is explained by the fact that the

young epithelial cells of the crypts move more rapidly to the surface, the greater superficies of the small intestine, so much increased by the villi, necessitating a greater supply of young cells to replace those that disintegrate on the surface; the elaboration of mucus often does not take place in the crypts, but first begins in the cells on the villi. In the large intestine, where the villi are absent, the passage to the surface takes place slowly, and the cells have time to produce secretion while they are within the crypts. Out of this arose the misconception that the crypts of the small intestine produced a serous fluid; those of the large intestine a mucoid secretion.

Between the epithelial cells migratory leucocytes from the underlying tunica propria are found in varying numbers.

FIG. 140.—LONGITUDINAL SECTION THROUGH THE APEX OF A VILLUS OF DOG. $\times 360$. The goblet-cells contain the less mucus the nearer they lie to the summit of the villus. Techn. No. 106.

The *tunica propria* consists chiefly of fibrillated and reticular connective tissue which contains an extremely variable number of leucocytes. Owing to the numerous crypts present the tunica propria of the large intestine is confined to the spaces between, and to a narrow zone below, the tubules, as in the stomach; throughout the small intestine the tunica propria extends into the villi.

The *muscularis mucosæ* consists of an inner circular and an outer longitudinal layer of smooth muscle-fibers. Fibers derived from the muscularis

mucosæ extend within each villus nearly to its apex. Their contraction effects a shortening of the villus.

The *submucosa* consists of loose fibrous connective tissue, and in the upper half of the duodenum contains branched tubular glands—the *glands of Brunner*. The excretory ducts of these glands are clothed with columnar cells, pierce the muscularis mucosæ, and run in the tunica propria parallel with the crypts of Lieberkühn. The walls of the tubules are formed of columnar gland-cells and a structureless *membrana propria*.

THE LYMPH-NODULES.

It has been previously mentioned that the tunica propria of the mucous membrane contains leucocytes or lymphoid cells in variable numbers, occurring

FIG. 141.—LONGITUDINAL SECTION THROUGH THE DUODENUM OF CAT. $\times 30$. The epithelium has become loosened from the connective tissue of the villus on the extreme left. The two villi at the extreme right are cut obliquely. The epithelium has fallen from the middle villus, so that the connective-tissue core lies exposed. The serosa is represented by a line beneath the longitudinal layer of the muscular coat. Techn. No. 103.

either as diffuse adenoid tissue or as circumscribed masses 0.5 to 2 mm. in size. The latter are lymph-nodes which occur singly as the *solitary nodules* (solitary follicles) or in groups as *Peyer's patches*.

The *solitary nodules* vary greatly in number in the gastric mucous membrane; they are more numerous in the intestines. They usually possess an oval form, and in the beginning of their development always lie in the tunica propria, close under the epithelium, with their base directed toward the muscularis mucosæ. With advancing growth (in cats at birth) they break through the muscularis mucosæ and expand in the submucosa, where the loose tissue offers but little resistance. The part of the nodule lying in the submucosa has a spherical outline, and soon becomes considerably larger than that within the tunica propria. The completed solitary nodules, therefore, are in general pear-

shaped, with the small end turned toward the epithelium. Where the nodules are situated the villi are wanting and the crypts are pushed aside. The solitary nodules are composed of adenoid tissue and usually contain a germinal center. The young leucocytes formed in them pass in part into the neighboring

FIG. 142.—TRANSVERSE SECTION OF A PATCH OF PEYER OF THE SMALL INTESTINE OF CAT. The crests of four nodules were not within the plane of the section. $\times 10$. Techn. No. 107.

FIG. 143.—FROM A SECTION OF THE SMALL INTESTINE OF A SEVEN-DAYS'-OLD KITTEN. $\times 250$. Crest of a solitary follicle. The epithelium on the left contains many wandering leucocytes. The epithelium on the right contains but three leucocytes. Techn. No. 107.

lymph-vessels, and in part wander through the epithelium into the intestine. The columnar epithelium covering the apex of the nodules contains wandering leucocytes (Fig. 143).

The patches of Peyer are groups of ten to sixty nodules that lie side by side, never over one another, each of which has the structure of a solitary

nodule. Occasionally the outline of an individual nodule is altered by the pressure of adjacent nodules (Fig. 142). They occur principally in the lower portion of the small intestine, and are either isolated from one another or embedded in diffuse adenoid tissue, in which case only the germinal centers can be distinguished. This is not infrequently the case in the vermiform process of man.

The *muscular layer* of the intestine consists of an inner robust circular, and an outer thinner longitudinal stratum of smooth muscle-fibers. In the large intestine the longitudinal muscular layer is only well developed at the folds corresponding to the intervals between the sacculi; between these folds it is extremely thin.

The structure of the *serosa* will be described with the Peritoneum.

FIG. 144.—FROM A CROSS-SECTION OF AN INJECTED SMALL INTESTINE OF RABBIT. $\times 50$. The lymph-nodule is sectioned so that in the upper half the superficial capillary network is visible, in the lower half, the capillary loops occurring within the interior of the nodule. The section is thick and unstained, and the crypts of Lieberkühn cannot be distinguished. 1. The network of blood-vessels within the muscularis; 2, within the submucosa; 3, within the tunica propria. Techn. No. 110.

THE BLOOD-VESSELS OF THE STOMACH AND INTESTINES.

The blood-vessels of the stomach and the large intestine have a precisely similar distribution, which is modified in the small intestine by the presence of the villi. In the stomach and the large intestine the entering arteries first give off small branches to the serosa, then pierce the muscularis, which they also supply, and form in the submucosa a network extending parallel to the surface. From this small twigs ascend through the muscularis mucosæ, and in the tunica propria at the base of the glands form another network parallel to the surface. Fine capillaries (4.5 to $9\ \mu$ wide) arise from the latter, and form

plexuses surrounding the gland-tubules and crypts; wider capillaries (9 to 18 μ) form a subepithelial plexus, which lies wreath-like about the mouths of the glands. Venules take their origin from the wide capillaries, pass vertically down between the gland-tubules, and open into a venous plexus lying parallel to the surface in the tunica propria; in their further course the veins run alongside the arteries. The veins arising from the venous plexus in the submucosa are furnished with valves to the point where they meet the veins of the small intestine approaching along parallel paths. The larger branches and the trunk of the portal vein are without valves.

In the small intestine only the arteries supplying the crypts are distributed in the same manner as in the large intestine. A special artery passes to the base of each villus (more than one when the villus is wide), breaks up into a capillary network extending beneath the epithelium, and terminates in a venous stem which descends almost vertically to the mucosa, taking up in its course the capillaries from the crypts. In the dog the artery enters the villus alongside the vein; it then breaks up into a subepithelial capillary plexus, that passes vertically or obliquely to the long axis of the villus over into the vein. The further course of the veins is the same as in the large intestine.

The duodenal glands (glands of Brunner) are enveloped in a capillary plexus supplied by the blood-vessels of the submucosa.

The lymph-nodes are surrounded by a superficial capillary network, from which fine capillaries extend into the interior; often these do not penetrate to the center, which is then without blood-vessels (Fig. 144).

THE LYMPH-VESSELS OF THE STOMACH AND THE INTESTINES.

The lymph- (chyle) vessels of the stomach and the large intestine begin in the mucous membrane as blind capillaries about 30 μ wide, and descend between the gland-follicles. In the mucous membrane of the small intestine the lymph-vessels begin in the axes of the villi; in cylindrical villi they are simple (in leaf-shaped villi multiple) canals (27 to 36 μ wide) closed at their upper ends—lymph-radicles or lacteals. All these vessels descend to a narrow-meshed capillary plexus lying at the base of the glands and extending parallel to the surface, which communicates by numerous anastomoses with a wide-meshed plexus in the submucosa; the lymph-vessels proceeding from this network penetrate the muscular coat and take up the vessels of a plexus lying between the circular and the longitudinal muscular strata, called the intramuscular lymphatic plexus, which takes up the lymph-capillaries of both muscular layers. The vessels then run beneath the serosa to the edge of the mesentery and pass onward between its folds. Many of the vessels are provided with valves.

In certain localities the course of the lymph-vessels in the mucosa is modified; the nodules of the patches of Peyer never contain lymph-vessels. They press aside the capillaries, which run in the interstices between them, constantly decreasing in number but increasing in caliber. It is probable that the lymph-

sinuses of the rabbit (p. 97) are nothing else than immensely widened, flattened capillaries.

THE NERVES OF THE STOMACH AND THE INTESTINES.

The numerous nerves, consisting mainly of gray fibers, form a plexus beneath the serosa, then penetrate the longitudinal layer of the muscular tunic and between this and the circular layer are arranged in a conspicuous network, the *intramuscular ganglionic plexus* or *Auerbach's plexus*; numerous groups of multipolar ganglion-cells are found along the course of the nerves, usually at the nodal points of the network, the meshes of which are angular or elliptical. From this network bundles of pale fibers are given off, usually at right angles, which in part supply the longitudinal and the circular strata of the muscular tunic, while another portion pierces the latter and enters the submucosa. In the muscu-

FIG. 145.—A. SURFACE VIEW OF AUERBACH'S PLEXUS OF INFANT. $\times 50$. *g*. Groups of ganglion-cells; *r*, layer of circular muscle-fibers, recognized by their rod-shaped nuclei. Techn. No. 111 a.
B. SURFACE VIEW OF MEISSNER'S PLEXUS OF THE SAME INFANT. $\times 50$. *g*. Groups of ganglion-cells; *b*, blood-vessel shimmering through the overlying tissue. Techn. No. 111 b.

lar coat the nerves form a rich rectangular-meshed network, from which nerve-fibers turn aside and after repeated division approach the muscle-fibers, on which (not within) they terminate in free club-shaped endings. The nerves in the submucosa form a delicate plexus, *Meissner's plexus*, whose meshes are narrower and whose groups of ganglion-cells are smaller. From this spring numerous fibers which enter the tunica propria, and in part weave a nervous net about the crypts, and in part enter the villi, where they terminate free in the parenchyma or close beneath the epithelium, without connection with the epithelial cells.

A network corresponding to the intramuscular ganglionic plexus occurs between the layers of the muscular coat of the esophagus.

THE SALIVARY GLANDS.

The salivary glands are the submaxillary, the sublingual, the parotid, and the pancreas. They are compound tubular glands, which elaborate either a mucoid or a serous fluid rich in albumin, or both the mucoid and the serous secretion. Accordingly we distinguish: (1) *mucous salivary glands* (sublingual in man, the rabbit, dog, and cat; submaxillary in the dog and cat); (2) *serous salivary glands* (the parotid in man, the rabbit, dog, and cat; submaxillary in the rabbit, and the pancreas); (3) *mixed salivary glands* (submaxillary in man, the ape, guinea-pig, and mouse).

The Sublingual Gland.—The excretory duct (duct of Bartholin) consists of a two-layered cylindrical epithelium and fibro-elastic tissue. It is continued as the intralobular or mucous tubes, whose low columnar epithelium exhibits the characteristic striation only in a few places. Intercalated tubules cannot be demonstrated with certainty, and it is much more probable that the mucous tubes pass directly into the terminal compartments. The latter are composed of a membrana propria and of gland-cells. The membrana propria is formed by stellate connective-tissue cells; the empty glandular cells occur in groups, and the “demilunes” therefore appear very large. The connective tissue between the tubules and the lobules is rich in leucocytes (Fig. 146).

The Parotid Gland.—The excretory duct (duct of Stenson) is distinguished by its broad, compact membrana propria close beneath the epithelium, but is otherwise like that of the sublingual gland. It divides and passes into the *intralobular* tubes, whose columnar cells exhibit at the base distinct vertical striation. Following these are the intermediate tubules, which are lined by elongated, often spindle-shaped cells. The intermediate tubules continue into the terminal compartments, which consist of a delicate membrana propria of stellate connective-tissue cells and of cubical serous glandular cells. In a condition of exhaustion the cells are small, dark, and granular; in a condition of activity they appear larger and somewhat lighter.

The Submaxillary Gland.—The excretory duct (duct of Wharton) possesses likewise a two-layered columnar epithelium, a connective-tissue layer rich in cells, and outside of this a thin stratum of longitudinally-disposed muscle-fibers; it continues as the intralobular tubes lined with characteristic “rodged” epithelium, which pass into the short intermediate tubules clothed with cubical cells. The latter lead into the acini, which are clothed with

FIG. 146.—FROM A THIN CROSS-SECTION OF HUMAN SUBLINGUAL GLAND. $\times 240$. Of the seven tubules represented, only three (1, 2, 3) are sectioned so as to be suitable for study. In 2 are six cells loaded with secretion (s.g.); and two empty cells (s.f.) are crowded to the periphery, where they form a “crescent.” In 3 all the cells are filled with secretion, and have deeply stained contents: 4, tangential section of a similar tubule. 5, 6, 7. Oblique sections of tubules like 1 and 2, which show the crescents, but not the lumen of the gland. mp. Membrana propria. b. Connective tissue with numerous leucocytes. z. Techn. No. 112.

either serous gland-cells (as in the parotid) or with mucous gland-cells and demilunes.

The Pancreas.—The excretory duct (duct of Wirsung and Santorini) is formed of a simple columnar epithelium and fibrous connective tissue, which latter is denser beneath the epithelium, looser toward the periphery. The walls

FIG. 147.—FROM A THIN SECTION OF HUMAN PAROTID GLAND. $\times 240$. *s*, Intercalated tubule; the outlines of the cells cannot be distinguished. The very narrow lumen of the gland-tubule is seen only at *t*; the remaining gland-tubules are cut obliquely. Techn. No. 112.

FIG. 148.—FROM A THIN SECTION OF HUMAN SUBMAXILLARY GLAND. $\times 240$. *A*, Cross-section of salivary tube: the epithelial-cells on the right are partially loosened from the surrounding connective tissue, *b*; on the same side the striation in the outer zone of the cells is best seen; *k*, nuclei of wandering leucocytes; *s*, secretion. *B*, Tubules (*m*) with mucous gland-cells showing four lumina; *e*, tubules with serous gland-cells showing one lumen; *b*, blood-vessels, of which the lowermost is cut longitudinally and contains colored blood-corpuscles. Techn. No. 112.

of the main excretory duct and its larger branches contain minute mucous glands. Intralobular tubes with their characteristic epithelium are wanting. The branches of the excretory duct continue directly into the intermediate divisions. The columnar epithelial cells of the former steadily diminish in height and

FIG. 149.—*A*, GLAND-CELLS OF PANCREAS OF CAT. $\times 560$. Above, groups of cells as they usually appear; below, two isolated cells. *B*, FROM A CROSS-SECTION OF PANCREAS OF AN INFANT. $\times 240$. Techn. No. 113.

eventually pass over into the flattened cells, placed parallel to the long axis, of the intermediate tubules. These tubules are very long and narrow; toward the acini they divide and then terminate abruptly.

The epithelium of the acini is composed of short cylindrical or conical cells, which are characterized by the highly refracting granules—"zymogen

granules"—occupying the zone adjoining the lumen, and are thus distinguished from all other glandular cells (Fig. 149 *A*). The clearer peripheral zone contains the round nucleus. The granular and clear divisions of the cell vary in proportionate extent with the functional condition of the cell. In the beginning of digestion the granules disappear and the clear belt becomes deeper. Subsequently the granular zone increases to such an extent that it occupies nearly the whole of the cell. In a fasting condition the two zones are of equal size.

In glands treated by the method of Golgi, the secretion often stains and the duct-system in its entire extent appears black. It may then be seen that striæ radiate out from the central lumen, but do not quite extend to the membrana propria; they branch, and without anastomosing terminate in free ends. They must not, without further consideration, be compared with the secretory capillaries of the parietal-cells, for these form a network embracing the cell, while here, at the most, the striæ, not their branches, lie upon the gland-cells; the latter lie rather within the cell and, in my opinion, indicate the residue of elaborated secretion that remains in the otherwise empty cell (also in the submaxillary, in the "demilunes"). (Fig. 150 and Fig. 151.)

FIG. 150.—FROM A SECTION OF PANCREAS OF ADULT MAN. $\times 320$. Techn. No. 119.

FIG. 151.—FROM A SECTION OF SUBMAXILLARY GLAND OF DOG. $\times 320$. Techn. No. 119.

The *blood-vessels* of the salivary glands are richly developed. The arterial stems run, as a rule, along the main excretory duct, divide into numerous branches which pass between the lobules and finally penetrate within the latter, where they break up into capillaries and form close networks embracing the tubules. The capillaries lie in immediate proximity to the gland-cells. The larger veins follow the course of the arteries.

With regard to the lymph-vessels little is known with certainty. The interfascicular clefts between the lobules and the tubules have been described as lymph-channels.

The salivary glands are profusely supplied with medullated and nonmedullated nerves, along the course of which microscopic groups of ganglion-cells occur. The fine medullated nerve-fibers form networks around the gland-tubules, but do not penetrate them, and ramify in the walls of the blood-vessels.

THE LIVER.

The liver is a compound tubular gland. On making an incision into a liver or on examining its outer surface, it will be observed that it is divided into irregular polygonal areas, well defined, as in the hog, or poorly defined, as in man and the majority of mammals. These areas are the *lobules* of the liver (incorrectly named *acini*). Their real form is somewhat like that of a prism with a rounded upper end and a transversely-blunted base (Fig. 152). They are 2 mm. high and 1 mm. broad. Close under the capsule of the liver the lobules are often arranged with their apices looking toward the surface, and a section taken parallel to the surface will pass through the lobules transversely (Fig. 154); in the interior of the liver the lobules extend in different direc-

FIG. 152.—SCHEME OF A HEPATIC LOBULE, represented in transverse section below and, by partial leveling, in longitudinal section above. In the left half the blood-vessels are drawn; in the right half only the cords of hepatic cells.
x 20.

tions. Each lobule consists of gland-cells and blood-vessels and is separated from neighboring lobules by the *interlobular* connective tissue—the *capsule of Glisson*—which supports the branches of the excretory duct—the hepatic duct—the branches of the portal vein and the hepatic artery, the lymph-vessels and the nerves. The demarcation of the lobules depends on the development of the interlobular connective tissue.

The main excretory duct, the *hepatic duct*, and its larger branches are composed of a single stratum of columnar epithelium, occasionally containing goblet-cells, and of fibrous connective tissue separated into a tunica propria and submucosa. The tunica propria contains glands, chiefly short pear-shaped follicles lined with mucous gland-cells, and also

isolated longitudinally- and transversely-disposed plain muscle-fibers.

The *cystic duct*, the *ductus choledochus*, and the *gall-bladder* exhibit the same structure; the tunica propria is elevated into minute anastomosing rugæ, and the mucosa is supplemented by a thin layer of smooth interlacing muscle-fibers. The columnar epithelial cells of the gall-bladder are distinguished by their height (0.05 mm.) from those of the ductus choledochus (0.024 mm.). The *vasa aberrantia* or blind bile-ducts, that occur chiefly at the left border of the liver, at the portal fissure, and surrounding the vena cava, are embryonal remains of liver-substance (isolated bile-ducts) and do not occur in the parenchyma of the organ. The branches of the hepatic duct—*interlobular bile-ducts*—exhibit thinner walls as they diminish in caliber; the larger are composed of simple columnar epithelium and fibro-elastic tissue; the small-

est possess only a structureless membrana propria and a simple layer of low epithelial cells, often showing a cuticular border, which as they enter at the margin of the lobules annex themselves directly to the true glandular cells. This transition is very difficult to see, and can only be distinctly perceived in sections in which the bile-ducts have been injected or blackened by Golgi's silver method.

FIG. 153.—LIVER-CELLS OF MAN. $\times 560$. A. Isolated liver-cells containing smaller and larger fat-drops, *f*; *b*, impression resulting from a blood-vessel. Techn. No. 114.
B. From a section: 1. Exhausted cells; 2. active cells, filled with secretion. Techn. No. 116.

The glandular-cells of the liver, the *hepatic cells*, are irregular polyhedral elements consisting of a granular protoplasm and of one or more nuclei; they have no cell-membrane. The protoplasm contains granules of pigment and globules of fat of various sizes. The cells vary in size from 18 to 26 μ . The

FIG. 154.—FROM A HORIZONTAL SECTION OF HUMAN LIVER. $\times 40$. Three central veins, cut transversely, represent each a center of as many hepatic lobules, which at the periphery are but slightly defined from their neighbors. Below and to the right of the section the lobules are cut obliquely and their boundaries cannot be distinguished. Techn. No. 116.

appearance of the hepatic cells depends, as in other glands, on the phase of functional activity. In a fasting condition they are small and dark, and have indistinct contours; during digestion they are larger, have a clear center, and at the periphery a coarsely-granular zone. In man the two conditions are frequently exhibited in the same liver (Fig. 153 B).

In the lower vertebrates (amphibians and reptiles) the hepatic cells form typical tubes, but in the higher vertebrates their arrangement is a very peculiar one, and not a trace of tubular structure is to be seen, as might be presupposed from the tubular character of the liver. The cells are united in small trabeculae or cords, the so-called *cords of cells*, which are radially disposed around a small vein (the central vein) situated in the axis of the lobule, and by lateral branches anastomose with neighboring cords of cells. A lumen cannot be distinguished in sections prepared by the usual methods: it can only be shown by injecting the system of canals from the hepatic duct or by employing Golgi's method, which blackens the bile. In such preparations it may be seen that

FIG. 155.—TRANSVERSE SECTION OF LIVER OF DOG. $\times 240$. Bile-capillaries blackened according to the method of Golgi. Techn No. 119.

the minutest interlobular bile-ducts are continued directly into the lobules, where they form, apparently, a network with polygonal meshes. In reality there are but few true meshes; the network is simulated by the zigzag course of the bile-canalculi and the crossing in different planes of the blind lateral twigs with which they are furnished (Fig. 155).

The ramifications of the intralobular system of canaliculi appear to have little relation to the branching of the cords of hepatic cells. The latter branch much less than the former and thus, apparently, the intralobular canaliculi have attained a certain degree of independence, as implied in the name *bile-capillaries* bestowed upon them. This also accounts for the hitherto always fruitless

endeavor to demonstrate a special wall for the bile-capillaries. There can be no wall other than that formed by a modification of the exoplasm of that side of the hepatic cells at which the capillary is situated.

Thin sections show clearly that the bile-capillaries stand in the same relation to the hepatic cells as the lumina of other glands do to the surrounding gland-cells, at least in most cases. But nevertheless certain differences exist. The first difference is this, that only a few, usually two, hepatic cells bound the bile-capillaries, while in other glands the lumen is surrounded by several cells. The explanation of this may be found in the conspicuous difference between the diameter of the lumen (bile-capillary) and that of the hepatic cell; two cells are sufficient to completely surround the lumen. The capillary is thus formed by the apposed furrow-like depressions of two contiguous hepatic cells (Fig. 162). A second difference consists in the relation of the surfaces of the hepatic cells to the bile-capillaries; they are in contact with the bile-capillaries not only on one, but on several surfaces. This momentarily confusing fact, though not a frequent, is nevertheless not an isolated phenomenon. One need but recall the relations in the fundus glands, where lateral twigs leave the chief lumen, branch and form a complete basket-work of fine canals embracing the parietal-cells, and where each parietal-cell presents not only one but all surfaces to the gland-lumen; but there the phenomenon is not so striking, because the branching off of the small lateral canals from the main lumen is easily recognized; in the liver the lateral

branches of the bile-capillaries are of the same diameter as the main lumen and are often of considerable length, subdivide, and may even anastomose directly with neighboring bile-capillaries, although this does not commonly occur, and thus every possibility of distinguishing between bile-capillaries, main lumen, and lateral canals vanishes. The fact that the hepatic cells are in contact with the bile-capillaries not only on one but on several surfaces, renders comprehensible the luxuriant ramification of the latter, despite the fact that few cells are required to circumscribe them.

Not infrequently it may be seen that short fine lateral twigs leave the bile-capillaries and terminate in a minute knob-shaped end. The knob corresponds to a small vacuole in the liver-cell, which communicates with the bile-capillary by means of the minute lateral twig. These are undoubtedly transient formations occurring in connection with certain functional phases; the proof of this I detect therein that entire areas of the system of canaliculi may be free from these knobs, while close beside every capillary is beset with them (Fig. 156).

Of the *blood-vessels* of the liver, the portal vein assumes the rôle that falls to the artery in other glands, while the hepatic artery is assigned the subordi-

FIG. 156.—FROM A SECTION OF LIVER OF DOG. $\times 450$.
Techn. No. 119.

nate part of the maintenance of the interlobular branches of the bile-ducts, of the portal vein, and of the nerves.

From the branches of the portal vein, which because they run in the interlobular connective tissue are called *interlobular veins*, spring numerous capillaries, possessing a width of 10 to 14 μ . They penetrate within the lobules, anastomose repeatedly during their course, and finally empty into a small vein lying in the axis of the lobule, the *central (intralobular) vein* visible in transverse and longitudinal sections even in the uninjected liver (Fig. 154). The central veins represent the radicles of the hepatic veins and empty into the *sublobular veins*, which run along the slightly-flattened side, the so-called base, of the hepatic lobules (Fig. 159).

FIG. 157.—HORIZONTAL SECTION OF LIVER OF RABBIT. Injected through the portal vein. $\times 40$. Three hepatic lobules are represented. The injection mass filled only the branches of the portal vein (interlobular veins); in the upper lobule it penetrated to the central vein. Techn. No. 118.

FIG. 158.—HORIZONTAL SECTION OF LIVER OF CAT. Injected through vena cava inferior. $\times 40$. Four hepatic lobules are shown. The injection mass filled the central vein and the capillaries emptying into it, but did not penetrate to the interlobular veins. Techn. No. 118.

The relations between the portal capillaries on the one side and the hepatic cells and bile-capillaries on the other calls for especial consideration. Between the meshes of the portal-capillary network lie the cords of hepatic cells, and the relation of the blood-vessels and gland-cells is consequently a very intimate one; sections show that a hepatic cell is in contact with capillaries, not only on one but on several sides (Fig. 160). This is a peculiar phenomenon; it does not occur in other glands, in which the blood-vessels touch the cells only at one surface, and is only comprehensible when we recall that in cross-sections the lumen (bile-capillary) is bounded by two cells, while in other glands the lumen is bounded by six or more cells (Fig. 161). But as in other glands, so also in the liver, the cells are inserted between the lumen on

the one hand and the blood-vessels on the other. Nowhere do blood-capillaries and bile-capillaries lie close beside one another; they are always separated by an intervening portion of the cell. The most convincing demonstration of this is afforded by thin sections of rabbit's liver, in which

FIG. 159.—FROM A VERTICAL SECTION OF LIVER OF CAT. Injection through vena cava inferior. A sublobular vein cut longitudinally; it takes up the central veins. The greater part of the injection mass has fallen out of the wide blood-vessels. $\times 15$. Techn. No. 118.

the blood-vessels have been cut transversely; these show plainly that the bile-capillaries run along the surfaces, the vascular capillaries at the corners of the hepatic cells (Fig. 162); however, this is not invariably the case; the bile-

FIG. 160.—FROM A SECTION OF LIVER OF RABBIT. $\times 240$. The portal-capillaries were injected with a red mass, the bile-capillaries with a blue mass. The hepatic cells are in contact with the blood-capillaries on both sides. At a few points the red mass has retracted and given rise to a space (*l*), between the hepatic cells and portal-capillaries. The bile-capillaries are nowhere in contact with portal-capillaries but are always separated from them by half the breadth of a cell. The dark spots on the portal-capillaries are optical cross-sections of blood-capillaries which run vertically to the plane of the section.

capillaries sometimes run along the edges, a disposition that occurs especially in man (Fig. 162 X).

The branches of the hepatic artery follow the course of the portal vein and ramify only in the interlobular tissue; they form capillary networks about

the larger bile-ducts, the branches of the portal and the hepatic veins. These capillaries are taken up by the portal interlobular veins or by the portal capillaries at the margin of the lobules. In the capsule of the liver the hepatic artery forms a wide-meshed capillary plexus. The course of the blood-vessels

FIG. 161.—SCHEME OF AN ORDINARY GLAND-TUBULE (LEFT) AND OF A HEPATIC TUBULE (RIGHT).

is therefore as follows: The portal vein enters at the transverse fissure, divides repeatedly into branches that steadily decrease in size and run in the connective tissue between the lobules as the interlobular veins; these break up into capillaries which pass toward the axis of the lobule and terminate in the central vein.

FIG. 162.—THIN SECTION OF LIVER OF RABBIT, WITH INJECTED BILE-CAPILLARIES. $\times 560$. (The drawing is *not* schematic.) Two of the hepatic cells are in contact with four blood-capillaries (1, 2, 3, 4). X. Bile-capillary at the edge of a hepatic cell.

Several of the latter unite in the formation of each of the sublobular veins, which, like the larger hepatic veins they form by their union, run between the lobules.

The *capsule* of the liver is composed of fibro-elastic tissue, which is especially well developed at the transverse fissure, where it is called the capsule of

Glisson, and in the form of special sheaths for the different channels penetrates the interior of the liver, where it is usually found in such small amounts between the lobules that the boundaries of the latter are very imperfectly defined. The walls of the veins are firmly attached to the liver substance by the interlobular connective tissue, and for this reason do not collapse when cut. Delicate fibers ("lattice-fibers") derived from the interlobular connective tissue penetrate into the interior of the lobule, where they are arranged in the form of a delicate, radially-placed "lattice-work."

The *lymph-vessels* accompany the branches of the portal vein, which they embrace in their ramifications; with the portal capillaries they enter the interior of the hepatic lobules, accompany them close up to the central vein, then pursue a divergent course. The deep lymphatics communicate with a superficial network of lymph-vessels, which occur in the capsule.

The nerves consist largely of nonmedullated fibers with which a few medullated nerve-fibers are mingled; they enter the interior of the liver in company with the hepatic artery and follow its ramifications; the exact mode of their termination is unknown. Ganglion-cells occur along the course of the nerves.

FIG. 164.—SCHEME OF A SYSTEM OF EXCRETORY CHANNELS ("DUCT-SYSTEM")

many points on the surface. The accompanying schematic representations may serve to elucidate the relations of the lobules. Imagine a system of ducts; alongside the excretory duct an artery, whose capillaries surround the terminal compartments and pass into a vein running along the base of the latter (Fig. 164).

FIG. 163.—FROM A SHAKEN SECTION OF HUMAN LIVER. $\times$ 240. *c.* Blood-capillaries, at *X* still containing blood-corpuscles. *b.* Interlobular connective tissue. On the right are five hepatic cells; the others have fallen out of the meshes of the capillary network. Techn. No. 117.

The secretion of the liver, the *bile*, frequently contains drops of fat, also granular masses of bile-pigment. Columnar cells from the bile-ducts are incidental admixtures.

That the structure of the liver really follows the type of the tubular glands, and that the cords of hepatic cells, with certain modifications, are comparable to the acini of other glands, the foregoing considerations have shown. The hepatic lobules, on the other hand, cannot without explanation be compared with the lobules of other glands; the latter, as a rule, consist of a duct-system, of which the excretory duct leaves the lobule at *one* place and continues into a larger duct.

In the hepatic lobules the ducts emerge at

FIG. 165.—SCHEME OF THE LIVER. Two lobules are shown, of which the left is only half carried out. The ramifications and anastomoses of the capillaries and the cords of hepatic cells have been omitted for the sake of clearness.

FIG. 166.—SCHEME OF TRANSVERSE SECTION OF LIVER. Four lobules represented. The separate systems of ducts are indicated by the difference in shading. A. Excretory ducts. E. Terminal compartments.

This is the principle of each of the many systems of ducts of which the liver consists; but there is one peculiarity: the somewhat tortuous terminal compartments extend in certain different directions (Fig. 165). At the base as well as above runs a vein, but—another variation—the vein takes up not only these capillaries but also those of the other side, where lies another system of ducts whose acini are in contact at the base with the same vein. The vein, therefore, lies in the axis of a complex or aggregation of terminal compartments, and such a complex is termed an hepatic lobule. (In the liver of the rabbit the central veins lie close under the surface, and only take up capillaries from one side.) If we now draw a comparison with the scheme Fig. 164, the artery corresponds to the portal vein in scheme Fig. 165, and the vein in Fig. 164 is the equivalent of the central vein of Fig. 165; one hepatic lobule corresponds not to one duct-system, but to parts of several systems. The simplicity of this schematic

FIG. 167.—FROM THE GREATER OMENTUM OF RABBIT. $\times 240$. The network is formed by large and small bundles of connective tissue. The wavy striation of the bundles can only be indistinctly seen, because the preparation was mounted in damar. At X the cells from the opposite surface can be seen shimmering through. Techn. No. 120.

presentation is based in part on the conception of well-defined lobules, as they occur in the hog. In other animals the distribution of the terminal ramifications is less regular; the latter bend into neighboring lobules, to which in part is owing their less distinct demarcation. Each system of ducts participates in the formation of several lobules.

THE PERITONEUM.

The peritoneum consists principally of bundles of fibrous connective tissue and numerous elastic networks; the free surface is covered by a simple layer of flat polygonal epithelial (endothelial) cells. The connection with subjacent parts (the parietes, the viscera, etc.) is effected by loose (subserous) connective tissue.

The connective-tissue bundles are arranged in thinner (in the visceral peritoneum) or thicker (in the parietal peritoneum, in the mesentery) layers

parallel to the surface, and interlace in various directions; in certain localities (in the greater omentum, in the middle of the lesser omentum) the bundles form a beautiful network with polygonal or rectangular meshes. The strands of the network are covered by plate-like epithelial cells (Fig. 167).

The number of connective-tissue cells among the fibrous bundles is on the whole not large; only in young animals are larger groups of cells found; they resemble plasma-cells and probably bear a close relation to the formation of blood-vessels.

The *elastic fibers* in the deeper layers of the peritoneum, particularly in the parietal portion, are profuse and vigorously developed.

The *subserous tissue* consists of loose connective tissue, many elastic fibers, and fat varying greatly in quantity; it is plentiful where the peritoneum is easily shifted over the underlying parts, but on the liver and the intestine it is so much reduced that it cannot be demonstrated as a special layer. At certain places, *e. g.*, in the broad ligaments, numerous bands of smooth muscle-fibers are found.

Blood-vessels and *nerves* are scantily represented; the latter terminate in part in Pacinian corpuscles.

Lymph-vessels occur in the superficial and the deeper layers of the peritoneum.

VI. THE RESPIRATORY ORGANS.

THE LARYNX.

The *mucous membrane* of the larynx is a continuation of the pharyngeal mucous membrane and like this is composed of an epithelium, a tunica propria, and a submucosa which binds the mucous membrane with underlying parts. The mucous membrane over nearly the whole of the organ is covered by a stratified ciliated columnar epithelium; the ciliary wave is directed toward the cavity of the pharynx. On the true vocal cords, on the anterior surfaces of the arytenoid cartilages and on the posterior surface of the epiglottis the epithelium is of the stratified scaly variety. The tunica propria consists of numerous elastic fibers and of white fibrous connective tissue, which in the lower animals is condensed to a membrana propria immediately beneath the epithelium. The tunica propria is the site of a varying number of leucocytes; in dogs and cats, solitary nodules are found in the mucous membrane of the ventricle of Morgagni. Papillæ occur mainly in the mucous membrane clothed with stratified squamous epithelium. The submucosa contains branched tubular mucous glands from 0.2 to 1 mm. in size.

The cartilages of the larynx are principally of the hyaline variety, which in a measure exhibit the peculiarities of the costal cartilages; to this belong the thyrioid, the cricoid, the greater portion of the arytenoid and often the

triteous cartilages. The epiglottis, the cartilages of Wrisberg and Santorini, the median portion of the thyroid, and the apex and vocal process of the arytenoid cartilages are of the yellow elastic variety. Occasionally the triteous cartilages are composed of white fibro-cartilage. Between the twentieth and thirtieth years of life ossification (chiefly endochondral) begins in the thyroid and the cricoid cartilages.

The larynx is richly supplied with *blood-vessels* and *nerves*. The blood-vessels form two or three networks in planes parallel to the surface, and a close subepithelial capillary plexus.

The *lymph-vessels* also form two communicating networks in horizontal planes, of which the superficial has the narrower channels and lies beneath the vascular capillary network.

The nerves in their course include microscopic ganglia. In part they terminate in end-bulbs and taste-buds. The latter are found on the posterior surface of the epiglottis.

THE TRACHEA.

The ciliated mucous membrane of the trachea possesses a structure like that in the larynx, excepting that the elastic fibers form a close network in which the fibers pursuing a longitudinal direction predominate. This network lies immediately beneath the epithelium and above the glands. The cartilages are of the hyaline variety. The posterior wall of the trachea is composed of a layer of transversely-arranged plain muscle-fibers, which is usually covered by a stratum of fibers extending longitudinally. The mucous glands of the posterior wall are distinguished by their size (2 mm.); they not infrequently penetrate the muscular layer, and lie in part in the fibrous tissue behind it.

The behavior of the blood-vessels, lymph-vessels, and nerves is the same as in the larynx.

THE BRONCHI AND THE LUNGS.

The lungs may be regarded as compound alveolar glands, in which, as in all glands, excretory and secretory (in this case respiratory) portions may be distinguished. The excretory division comprises the larynx, the trachea, and the bronchi. Each bronchus on entering the lung divides repeatedly and within the same undergoes continual subdivision, giving off small lateral twigs and branching at acute angles, with gradual decrease in the caliber of the branches, finally breaking up into minute twigs that nowhere anastomose with one another and that retain the characteristics of the bronchus to a diameter of 0.5 mm.

At this point the respiratory division begins. Isolated hemispherical evaginations, the *alveoli*, appear at irregular intervals on the walls of the minute bronchi. Such bronchi are called *respiratory* or *terminal bronchioles*. These divide and lead into the *alveolar ducts*, which differ from the terminal bronchioles only in the larger number of alveoli in their walls. The alveolar ducts divide at right or acute angles, and pass without sharp demarcation into

the slightly-expanded *terminal vesicles* (less correctly, infundibula), whose walls are thickly beset with alveoli.

The entire respiratory division is separated by areolar tissue into *lobules* 0.3 to 3 cm. in size. All the branches of the excretory division to a diameter of 1.5 to 1 mm. and less lie between the lobules—as “interlobular ducts.”

The *minute structure of the bronchi* in the largest branches does not differ from that of the trachea. Gradually, however, modifications appear, which first involve the cartilages and the musculature. The C-shaped ring cartilages

FIG. 168.—CROSS-SECTION OF BRONCHUS, TWO MILLIMETERS THICK, OF CHILD. $\times 30$. Techn. No. 123.

are replaced by irregular plates lying on all sides of the bronchial wall. They diminish in size and thickness with the decrease in the diameter of the bronchi and disappear altogether in bronchioles 1 mm. in diameter.

The *smooth muscle-fibers* are circularly disposed in a continuous layer lying within the cartilages and form a complete investment for the tube. The thickness of the muscular layer decreases with the diameter of the bronchi; but muscle-fibers are still found as far as the alveolar ducts. In the infundibula they are wanting.

The *mucous membrane* is thrown into longitudinal folds and consists of a stratified ciliated epithelium containing goblet-cells, which in the smaller bronchi becomes gradually reduced to a single stratum, and of a connective-tissue tunica propria. The latter contains numerous longitudinal networks of

FIG. 169.—FROM A SECTION OF LUNG OF ADULT MAN. $\times 50$. The terminal bronchiole divides into two branches (on the right). A portion of the wall of the bronchiole fell within the plane of the section. Above the entrance to the alveoli can be seen; below the alveoli are viewed from the side. The epithelium of the bronchiole is mixed. The epithelial lining of the alveoli is only partially visible with this magnification. Techn. No. 124.

elastic fibers and leucocytes in greatly varying numbers. Occasionally solitary nodules occur, from the crest of which leucocytes wander through the epithelium into the bronchial tubes.

FIG. 170.—FROM SECTIONS OF HUMAN LUNG (A AND B), AND (C), OF LUNG OF A KITTEN NINE DAYS OLD. $\times 240$. A. Mixed epithelium of terminal bronchiole. B and C. Alveoli drawn with change of focus. The margin of the alveolus is shaded; it can be seen that the epithelium covering it is like that in the base of the alveolus (the light portion); the nuclei of the cells are not visible. Techn. No. 124.

Branched tubular mucous glands occur as far as the cartilages extend; they are situated outside of the muscular layer (Fig. 168). They are numerous and do not disappear until at the beginning of the respiratory bronchioles.

External to the cartilages is a *fibro-elastic tunic* which envelops the entire bronchus including the accompanying vessels and nerves.

The *minute structure of the respiratory division*, after the gradual disappearance of the cartilages and glands, is distinguished especially by the nature of the epithelium.

The *respiratory bronchioles* following the smallest excretory bronchi still contain a single layer of ciliated columnar epithelium, but as they proceed the cilia are lost, the cells become cubical, and between these another kind of epithelial cells appears, in the form of thin nonnucleated plates of different sizes. These plates and isolated or small groups of cubical cells form an epithelium called *respiratory epithelium*. The transition of the cubical into the respiratory epithelium is not abrupt and sharply defined, but occurs in such wise that at one extremity of the bronchiole cubical, at the other extremity respiratory epithelium is found; or that groups of cubical cells are surrounded by respiratory epithelium or the reverse. The respiratory bronchioles contain, therefore, a mixed epithelium (Fig. 169 and Fig. 170 A).

The epithelium of the *alveolar ducts* and of the *alveoli* is the same as the respiratory epithelium of the bronchioles. The developmental history teaches that the smaller nonnucleated plates originate from cubical cells which become flattened by inspiration, that is, by the inflation of the alveoli. The larger plates are formed by the subsequent blending of several smaller ones. The alveoli of old embryos and in stillborn children contain only cubical cells. The walls of the alveolar ducts and the alveoli, in addition to the previously mentioned muscle-fibers in the former, are composed of a delicate fibrous framework and many elastic fibers. The latter are circularly arranged in the alveolar ducts, and encircle the entrance to the alveolus (the mouth or base); delicate fibers spring from this annular bundle and form a network surrounding and supporting the entire wall of the alveolus. The elastic rings of neighboring alveoli grow together at the points of contact and thus constitute the alveolar septa.

The areolar tissue between the lobules of the lung—the interlobular connective tissue—contains extremely fine elastic fibers and a few connective-tissue cells, and in the adult black pigment-granules and inhaled carbonaceous particles. In children the interlobular connective tissue is more richly developed and the demarcation of the lobules more distinct.

The surface of the lung is covered by the *visceral pleura*; this is composed of connective-tissue, numerous fine elastic fibers, and on its free surface is clothed by a simple stratum of flat polygonal epithelial (endothelial) cells. The *parietal pleura* has the same structure, but contains fewer elastic fibers.

The *blood-vessels of the lungs*, the branches of the pulmonary artery, enter at the hilus of the lung and run beside the bronchi, bronchioles, alveolar ducts, and between the infundibula, where they break up into a very narrow-meshed capillary network, placed immediately beneath the respiratory epithelium of the terminal bronchioles, the alveolar ducts, and the alveoli. The veins arise each at the base of an alveolus, and unite into branches that follow the bronchi and arteries. The walls of the bronchi are supplied by the bronchial arteries, which furnish a deep capillary plexus for the muscles and the glands, a superficial

plexus for the tunica propria. These capillaries are taken up in part by the bronchial veins, in part by the pulmonary veins.

Of the *lymphatic vessels* two groups are recognized, a well-developed superficial plexus beneath the pleura and a wide-meshed deep plexus in the interlobular connective tissue. From these networks small stems furnished with valves proceed, which follow the bronchi and emerge at the hilus, where they connect with the bronchial lymph-nodules.

The numerous nerves of the lungs, derived from the sympathetic and the vagus, contain medullated and nonmedullated nerve-fibers and small groups of ganglion-cells. The nerve-endings stand in especial relation to the walls of the blood-vessels.

FIG. 171.—FROM A SECTION OF LUNG OF CHILD, INJECTED THROUGH PULMONARY ARTERY. $\times 89$. Of the five alveoli drawn the three upper ones are fully injected. Techn. No. 126.

THE THYROID GLAND.

The thyroid body is a compound tubular gland, whose excretory canal, the thyro-glossal duct, opening at the foramen cecum of the tongue, with

FIG. 172.—A LOBULE FROM A THIN SECTION OF THYROID BODY OF ADULT MAN. $\times 220$. The tubules vary in diameter. Techn. No. 127.

the exception of a few atrophic remains was obliterated in the embryonic stages of the organ. It consists, therefore, of *completely closed tubules*, which are united into lobules by loose connective tissue. The tubules differ greatly in size (40 to 120 μ in diameter) and are lined by a simple layer of cubical epithelial cells. Their contents consists of a characteristic, homogeneous, vis-

cid mass, the colloid substance, which is found also in the lymph-vessels of the organ. The *blood-vessels* are exceptionally numerous, and break up into capillaries which form a network close beneath the epithelium. The *lymphatics*, likewise profuse, form a network lying between the tubules. The *nerves* follow the

FIG. 173.—SECTION OF SECONDARY LOBULES OF THYMUS BODY OF A SEVEN-DAYS'-OLD RABBIT. $\times 50$. The lower lobules are sectioned tangentially, so that chiefly only cortex is visible. Techn. No. 128.

ramifications of the blood-vessels and form networks distributed especially to the vascular walls, some of which also surround the gland-tubules. The penetration of the terminal twigs into the epithelium has not been observed.

THE THYMUS BODY.

The thymus body, in its first anlage an epithelial organ, consists in childhood of lobes 4 to 11 mm. large, which are enveloped by a fibrous connective-tissue sheath containing fine elastic fibers.

The capsule sends septa into the lobes, by which a subdivision into smaller (secondary) lobules, 1 mm. large, is effected. Each of the lobules consists of adenoid tissue, denser at the periphery than in the center, so that a darker cortical zone and a lighter medullary substance may be distinguished. In the medullary substance concentrically striated bodies, varying greatly in number and size (15 to 180 μ in diameter), are found; they are masses of

FIG. 174.—CORPUSCLE OF HASSALL FROM A SECTION OF THYMUS BODY OF A YOUNG DOG. $\times 50$. Techn. No. 128.

altered epithelial cells [the remains of the epithelial structures which in the embryonic stages constituted the principal bulk of the organ]. They are called *Hassall's corpuscles*.

The blood-vessels are richly developed and supply the cortex and the medulla with capillary networks. The lymphatics, likewise, are very numerous; the larger vessels lie on the surface of the organ, their branches run in the interlobular septa and penetrate into the medullary substance.

At a later period the tissue of the thymus undergoes retrogressive change; the greater part of the adenoid structures disappear and are replaced by fat.

VII. THE URINARY ORGANS.

THE KIDNEYS.

The kidneys are compound tubular glands, which consist exclusively of minute tubes, the *uriniferous tubules*. The macroscopically perceptible differences between the peripheral and central portions of the organ, the so-called *cortical* and *medullary* regions, are principally determined by the course of the uriniferous tubules, the divisions within the cortex pursuing a tortuous, those within the medulla a straight course.

Each *uriniferous tubule* begins in the cortex as a spherical dilatation, the Malpighian corpuscle, which is marked off by a constriction, the *neck*, from the greatly convoluted succeeding division, the *proximal convoluted tubule*. This passes into a straight portion, which is at first centrally directed, but soon turns back and forms a loop, *Henle's loop*, in which a *descending* and an *ascending limb* may be distinguished. The latter passes into a convoluted portion, the *intercalated tubule* (*irregular* and *distal convoluted portions*), that as it proceeds assumes a straight course, and is then called *collecting tubule* (Fig. 175). The collecting tubules, during their centrally-directed course, take up other distal convoluted tubules, unite under acute angles with neighboring collecting tubules, and converge toward the apex of a renal papilla, where, diminished in number but greatly increased in diameter, they terminate in the *papillary duct* or *duct of Bellini*, which opens on the free surface of the papilla. Henle's loop-tubules and the collecting tubules are named straight tubules (*tubuli recti*). Each uriniferous tubule pursues a completely isolated course until it is taken up by a collecting tubule. The loops of Henle and the peripheral portions of the collecting tubules are grouped into bundles as they pass toward the medulla, and form the familiar striae in the cortex known as *medullary rays* or *pyramids of Ferrein*.

The minute structure of the uriniferous tubules varies so greatly in the several divisions that a special consideration of each is necessary.

The *Malpighian corpuscles*, from 0.13 to 0.22 mm. in size, consist of a spherical mass of convoluted blood-vessels, the *glomerulus*, and the expanded and invaginated blind initial extremity of a uriniferous tubule, the *capsule of*

Bowman. The glomerulus lies within the invaginated portion of the capsule, and is almost completely enveloped by it. Accordingly, two layers are distinguished in the capsule of Bowman, an inner (quasi visceral) which lies close

FIG. 175.—SCHEME OF THE COURSE OF THE URINIFEROUS TUBULUS (LEFT) AND OF THE RENAL BLOOD-VESSELS (RIGHT), sketched from a section of kidney of an infant seven weeks old. $\times 10$. *R.* Cortex. *M.* Medulla. *m.s.* Medullary rays. l_1, l_2, l_3 . Three renal lobules. *a* Malpighian corpuscle; *b*, proximal convoluted tubule; *c*, descending, *d*, ascending limb of Henle's loop-tube; *e*, distal convoluted tubule; *f*, collecting tubule; *f_1*, portions of collecting tubules; *g*, excretory duct. 1. Branch of renal artery. 2. Interlobular artery. 3. Afferent artery. 4. Efferent artery. 5. Interlobular vein. 6. Branch of renal vein. *X* and *XX*. Branches supplying the medulla.

FIG. 176.—URINIFEROUS TUBULES OF A FOUR-WEEKS-OLD RABBIT, ISOLATED $\times 30$. *a*. Malpighian corpuscle. *b*. Proximal convoluted tubule. *c*. Henle's loop, descending limb; *d*, ascending limb. *f*. Collecting tubule. *g*. Papillary duct. Techn. No. 129.

upon the glomerulus, and an outer (quasi parietal) layer; the former, in young animals, is composed of cubical cells, which later become more and more flattened, the latter of flat polygonal cells (Fig. 178). At the neck of the capsule the outer layer passes over into the walls of the *proximal convoluted tubule*,

which is 40 to 60 μ thick. The cells in a condition of functional activity are tall and exhibit a clear central zone surrounding the nucleus, while the outer zone or base is striated, with the striae placed radially to the narrow lumen; in

FIG. 177.—FROM A SECTION OF HUMAN KIDNEY, INCLUDING A PORTION OF THE CORTX AND THE MEDULLA. At x two renal corpuscles have fallen out. $\times 20$. Techn. No. 130.

an exhausted condition the cells are lower, dim, their boundaries are indistinctly defined, and the free surface presents a striated border. Both stages of

FIG. 178.—SCHEME. On the left an artery which gives off an afferent vessel toward the right, this breaks up into branches, which turn into the radicles of the efferent vessel (directed toward the right). The three loops are intended to represent the glomerulus; this lies in Bowman's capsule, of which both layers are visible, below, the latter passes into the uriniferous tubule.

secretion occur simultaneously. The *descending limb* of Henle's loop is 9 to 15 μ thick, and the lumen is very wide; it is lined by squamous epithelial cells whose nuclei often protrude into the lumen. The *ascending limb* is 23 to 28 μ thick, and the lumen relatively narrower; the epithelial cells resemble those of the convoluted divisions, but are somewhat lower. The transition of the narrow descending limb into the thicker ascending portion does not always occur at the loop. The *intercalated* or *distal convoluted portion* is from 39 to 46 μ

thick, and the epithelial cells are cylindrical or conical in shape and have a peculiar luster. The *collecting tubules* increase in thickness as they approach the apex of the papilla; the thinnest have a diameter of 45 μ , the thickest (duct

of Bellini) of from 200 to 300 μ . Their epithelial cells are in part clear, in part granular columnar elements, which increase in height with the increase of the diameter of the tubule (Fig. 181, 3).

The uriniferous tubules are covered in their entire length by a structureless membrana propria situated outside of the epithelium, which is thickest in the descending limb of Henle's loop. The tubules are enveloped by a small

FIG. 179.—FROM A SECTION OF KIDNEY OF MOUSE. $\times 240$.

amount of loose connective tissue (interstitial connective tissue), which at the surface of the kidney becomes condensed and forms a fibrous investment containing plain muscle-fibers. The blood-vessels run in the interstitial connective tissue.

The blood-vessels of the kidneys. The renal artery divides in the hilus of the kidney into branches, which after giving off small twigs to the capsule and

FIG. 180.—A. ISOLATED CELL OF A PROXIMAL CONVOLUTED TUBULE. The base of the cell is separated into minute rods. B. Transverse section of a proximal convoluted tubule; the "rods" appear as delicate striae. Both preparations are from a cat's kidney. $\times 240$. Techn. No. 130.

FIG. 181.—FROM A TRANSVERSE SECTION OF THE MEDULLA OF HUMAN KIDNEY, THROUGH THE BASE OF A PAPILLA. $\times 240$. 1. Descending; 2. ascending limb of Henle's loop; 3. collecting tubule; 4. blood-vessel filled with blood-corpuscles. Techn. No. 130.

to the calices of the kidney, enter the parenchyma of the organ at the circumference of the papillæ, and without branching pass to the boundary between the cortex and the medulla. Here they bend at right angles and form arches with the convexity toward the periphery. From the convex side of the arches, at regular intervals, spring branches running toward the periphery; these are the

interlobular arteries,* which give off short lateral twigs, each one of which supplies the *afferent vessel* of a glomerulus. The glomeruli arise by the rapid division of the afferent artery into groups of convoluted capillaries, which reunite into a single vessel, the *efferent artery*, which is somewhat smaller than the entering vessel. The efferent artery breaks up into a capillary network with round meshes in

FIG. 182.—FROM A LONGITUDINAL SECTION OF INJECTED KIDNEY OF GUINEA-PIG. Techn. No. 133.

FIG. 183.—SECTION OF KIDNEY OF MOUSE. $\times 180$. Techn. No. 134.

the region of the convoluted tubules, with elongated meshes in the region of the medullary rays. From the latter the veins arise, *interlobular veins*, which lie

* Microscopic regions of the kidney, with ill-defined boundaries, in the axis of which lies a medullary ray, and at the periphery of which interlobular arteries ascend, are designated lobules. In Fig. 175 three lobules, l_1 , l_2 , l_3 , are indicated by dotted lines. These lobules have no relation whatever to the lobules of the kidney during fetal life.

close beside the interlobular arteries and follow them throughout their course. The vessels of the peripheral zone of the cortex converge to certain points, where they unite into radicles arranged in a stellate form, the *venæ stellatæ*, which join the interlobular veins (Fig. 175 and Fig. 182).

The medulla receives its blood supply from the *arteriæ rectæ*, which arise from the arterial arches at the juncture of the medulla and the cortex, from the efferent vessels of the most deeply situated—and also the largest—glomeruli, and direct from centrally-running branches of the interlobular arteries. The veins of the medulla take their origin from the wide-meshed capillary network surrounding the large excretory ducts and join the venous arches at the juncture of the medulla and the cortex.

The lymph-vessels run in part superficially, in the capsule, and in part accompany the arteries in the parenchyma of the organ. The nerves form

FIG. 184.—TRANSVERSE SECTION OF THE LOWER HALF OF HUMAN URETER. $\times 15$. *e*, Epithelium; *l*, tunica propria; *s*, submucosa; *l*, inner longitudinal muscle-bundles; *r*, circular layer of muscle-bundles; *l*₁, accessory outer longitudinal muscle-bundles. Techn. No. 135.

plexuses which surround the arteries as far as the Malpighian corpuscles; in connection with the uriniferous tubules no nerves have as yet been found.

THE URETERS.

The ureters, the calices, and the pelvis of the kidney are composed of three coats, the mucous membrane, which lies innermost, the muscular coat, and surrounding this the outer fibrous coat (Fig. 184).

The tunica propria of the *mucous membrane* consists of delicate connective-tissue fibers, which, richly interspersed with cellular elements, passes without sharp demarcation into the tissue of the submucosa. The epithelium covering the tunica propria is the so-called "*transitional epithelium*;" that is, a stratified scaly epithelium composed of but few layers, of which the uppermost layer consists of cylindrical or cubical, only slightly flattened elements. Occasionally,

instead of the latter, large plate-like cells are present, which contain several nuclei that have arisen by amitotic division.

The *muscular coat* consists of an inner longitudinal and an outer circular layer of smooth muscle-fibers, which in the lower half of the ureter possesses an additional discontinuous outer layer of longitudinally-arranged muscle-bundles.

The *fibrous coat* consists of loosely-united connective-tissue bundles.

The mucous membrane of the calices is continued over the surface of the renal papillæ, the circular muscle-fibers form a sphincter muscle around the papillæ.

Both the blood- and the lymph-vessels are especially numerous in the mucous coat. The nerves are distributed principally to the muscular coat; single fibers extend into the tunica propria as far as the epithelium.

FIG. 185.—PORTION OF A VERTICAL SECTION OF HUMAN VESICAL MUCOUS MEMBRANE. $\times 560$. Techn No. 136.

THE URINARY BLADDER.

The urinary bladder likewise consists of a mucous, a muscular, and a fibrous coat. The epithelium resembles that of the ureter and the pelvis of the kidney in every particular; a distinction from these is impossible. The tunica propria occasionally contains solitary lymph-nodules. The muscular coat consists of strata of smooth muscle-fibers, an inner and an outer longitudinal layer, which enclose between them a circular layer. The layers interlace in such a manner that it is not possible to define their exact limits. At the base of the bladder the inner longitudinal layer is augmented, the circular layer forms the not always distinct internal vesical sphincter. Blood- and lymph-vessels have the same distribution as in the ureter; microscopic groups of ganglion-cells are situated along the course of the nerves.

In the tunica propria of the lower division of the pelvis of the kidney, the upper portion of the ureter and the bladder round or oval bodies occur, which have been erroneously regarded as glands. They are sprouts of the surface epithelium, possess the same structure, are without a lumen, and occasionally have even severed their connection with the superficial epithelium.

THE URETHRA.

The *female urethra* is composed of a mucous coat and a robust muscular coat. The *tunica propria* consists of delicate fibrous connective tissue containing numerous connective-tissue cells, and is beset with papillæ, that are especially well developed near the meatus. The epithelium varies, in some individuals it is a stratified scaly, in others a simple columnar epithelium. A few branched simple tubular glands are present; they occur in small groups at the meatus, and are called "periurethral" glands. The muscular coat consists of an inner longitudinal and an outer circular layer of nonstriated muscle-fibers, between which extends a compact fibrous connective tissue containing many elastic fibers. The mucous coat is richly supplied with veins.

The *male urethra* (better, male urogenital sinus) is likewise composed of a mucous coat and a muscular coat, but they vary in structure in the different parts of the canal. In the prostatic portion the epithelium resembles that of the bladder; in the membranous division it gradually passes into the stratified columnar variety, which in the spongy part is transformed to a simple columnar epithelium. From the fossa navicularis on, the epithelium is of the stratified squamous type. The *tunica propria* is rich in elastic fibers and is beset with papillæ, that are especially well developed in the fossa navicularis. Isolated branched simple tubular glands (Littre's glands) occur throughout the entire urethra. The muscular coat, in the prostatic division, consists of an inner longitudinal and an outer circular layer of smooth muscle-fibers; both layers are still well defined in the membranous portion, but gradually cease in the spongy portion, where the circular layer, still conspicuous in the bulbous urethra, is the first to disappear; in the anterior part of the spongy division a few oblique and longitudinal bundles occur (Fig. 193). The mucous membrane has a rich vascular supply. The lymph-vessels lie beneath the blood-vessels.

VIII. THE REPRODUCTIVE ORGANS.

THE MALE REPRODUCTIVE ORGANS.

THE TESTICLE.

The testicles are compound tubular glands, which are enveloped in a connective-tissue capsule. This capsule, the *tunica albuginea*, is a tough membrane which encloses the parenchyma and on the posterior upper aspect is greatly thickened, forming a mass, the *corpus Highmori* or *mediastinum*, which juts into the interior of the organ. From this a number of septa arise, which pass along divergent paths to the tunica albuginea and so divide the parenchyma of the testicle into *pyramidal lobules*, with their base directed toward the capsule, their apex toward the corpus Highmori. The

tunica albuginea consists of dense fibrous connective tissue, which on its free surface (the visceral layer of the tunica vaginalis) is covered by a simple layer of flat epithelial cells, on its inner surface is in contact with a layer of loose connective tissue, which supports numerous blood-vessels and is called *tunica vasculosa*; the latter is connected with the interlobular septa. The corpus Highmori is a dense connective-tissue structure and encloses a network of freely-anastomosing tubules, the *rete testis*. The septa consist of bundles of connective tissue which are continuous with the connective tissue surrounding the individual seminiferous tubules. This "interstitial" connective tissue is rich in cellular elements, the so-called *interstitial cells*, which are in part flat

FIG. 186.—CROSS-SECTION OF TESTICLE OF A NEWBORN CHILD. $\times 10$. Techn. No. 140.

connective-tissue cells, in part spherical cells containing pigment or fatty granules.

The *seminiferous tubules* may be divided into three portions: they begin as (1) the *convoluted tubules*, which pass into (2) the *straight tubules*, which continue as (3) the *rete testis*. The convoluted tubules are round, winding canals, about $140\ \mu$ in diameter. Their initial extremity has not yet been definitely located; probably they are united with one another at the periphery, beneath the tunica vasculosa, and form a network from which numerous tubules turn aside and with many windings pass toward the corpus Highmori. Tubules with blind ends have been observed. During their course the tubules unite

with one another and diminish in number. Not far from the corpus Highmori the convoluted tubules pass into the straight tubules, which, considerably reduced in size (20 to 25 μ thick), after a short course penetrate into the mediastinum and form the rete testis, the tubules of which measure from 24 to 180 μ .

The walls of the *convoluted tubules* from without inward consist of (1) several layers of flattened endothelioid connective-tissue cells; (2) a thin *membrana propria*; and (3) of a stratified epithelium the character of which varies greatly in the several divisions of the tubules. When the gland is in a condition of rest several strata of spherical cells, whose nuclei stain more or less intensely, may be seen lining the tubules (Fig. 187). In a condition of activity the epithelium exhibits a cycle of phenomena relating to *spermatogenesis*. The cells lying next to the basement membrane—*parietal stratum*—are of two kinds: the *sustentacular cells* or *Sertoli's columns*, which take no direct part in the production of the seminal filaments, and the *spermatogenic cells*, the real

FIG. 187.—FROM A CROSS-SECTION OF TESTICLE OF OX. $\times 50$. In the processes of fixing and hardening the epithelium has become somewhat shrunken, so that spaces occur between it and the interstitial connective tissue. Techn. No. 141.

producers of the semen. They multiply by indirect division, and grow to be large cells, that occupy the next layer within. These are the *mother-cells*, which divide twice, each giving rise to four *daughter-cells* lying in a zone still nearer to the center of the tubule. The latter are the *spermatids* and from them the spermatozoa are directly derived. The nucleus of each spermatid develops into the head of a spermatozoon, a small portion of the protoplasm forming the caudal filament. The middle-piece reacts like paranuclein and probably is derived from the centrosome. While these changes are in progress the columns of Sertoli grow in length centrad and a large number of spermatids form a connection with each one of them; it is highly probable that by means of this connection the spermatids receive their nutritive material.

The walls of the *tubuli recti* consist of a *membrana propria* and within this of a simple layer of low columnar cells.

The canals of the *rete testis* are lined by a simple stratum of cubical or flat epithelial cells.

The *arteries* of the testicles are branches of the spermatic artery, which proceed in part from the mediastinum and in part from the tunica vasculosa to the intertubular septa, and then break up into capillary networks which surround the seminiferous tubules. The veins follow the course of the arteries. The *lymph-vessels* form a plexus beneath the tunica albuginea, which is in connection with a network of lymph-capillaries enveloping the seminiferous tubules. The *nerves* form networks about the blood-vessels, from which single fibers are said to branch off, pierce the membrana propria, and terminate in club-shaped endings between the epithelial cells.

FIG. 188.—CROSS-SECTION OF A SEMINIFEROUS TUBULE OF MOUSE. $\times 360$. Below on the left are seen the at first round nuclei of the spermatids, which become oval (above), and are transformed (below on the right) into the heads of the seminal filaments. Techn. No. 142.

THE SEMEN.

The secretion of the testicles, the semen, consists almost exclusively of *spermatozoa*, structures in which a *head* and a *tail* are distinguished. In man the *head* is 3 to 5 μ long and 2 to 3 μ broad, flattened, seen from the side pyriform in shape, with the narrow end directed forward, seen on its broadest surface oval, with the anterior end rounded. The *tail* when very highly magnified exhibits a delicate filament extending from end to end, the *axial fiber*, which is composed of delicate fibrils. Three divisions are recognized in the

tail: the *middle-piece*, lying next to the head, $6\ \mu$ long and scarce $1\ \mu$ broad; following this the *main-piece*, 40 to $60\ \mu$ long and gradually diminishing in thickness; the tip of the tail, the *end-piece*, is about $10\ \mu$ long and consists of the projecting axial fiber.

The different forms of spermatozoa in animals cannot be described here. In birds and tailed amphibians a spiral fiber, united to the axial fiber by a hyaline membrane, has been discovered; it has also been found in the rat and other mammals, but has not been demonstrated with certainty in man.

The spermatozoa are distinguished by their extraordinary vitality (probably due to the calcareous matters which they contain).

The vibratile movements of the spermatozoa are executed by the cilium alone, which propels the head before it; they seldom occur in the concentrated secretion of the testicle, and first begin only after dilution normally effected by admixture of the fluids of the ampullæ, of the seminal vesicles, of the prostate gland, and of Cowper's glands. In this mixture of fluids the motions may continue 24 to 48 hours after death, and for a still longer period in the secretions of the female generative tract. Water paralyzes the movement, which, however, may be stimulated to renewed activity by the addition of normal animal fluids of alkaline reaction and moderate concentration; normal fluids in general, also a one per cent. salt solution exert a favorable influence on the vibrations of the spermatozoa, while acids and metallic salts suspend them. In motionless spermatozoa the caudal filament is frequently looped (Fig. 189, 3).

FIG. 189.—1, 2, 3. HUMAN SPERMATIZOON. $\times 360$. 1. Viewed from the surface. 2. Viewed in profile. 3. Looped seminal filament. 4. Spermatozoon of ox; a, head; b, middle-piece; c, main-piece. The end-piece and the demarcation of these parts cannot be perceived with this magnification. Techn. No. 144.

THE EXCRETORY DUCTS OF THE TESTICLE.

The excretory ducts of the testicle include the epididymis, vas deferens, the seminal vesicles, and the ejaculatory duct. (The tubuli recti and rete testis belong to the excretory ducts, but were described with the gland because they are enclosed within it.) From the upper end of the rete testis about fifteen *vasa efferentia* emerge, which by their progressively-increasing convolutions form as many conical lobules, the *coni vasculosi*. The aggregate of the coni constitute the *globus major* of the epididymis. By the union of the *vasa efferentia* the *vas epididymis* arises, which by its complex convolutions forms the body and *globus minor* of the epididymis, and then continues as the *vas deferens*.

The *vasa efferentia* are lined by an epithelium consisting of totally dissimilar varieties; groups of simple ciliated cylindrical elements alternate with clusters of cubical cells without cilia; the latter consequently have the

appearance of simple saccular glands, which, however, do not produce evaginations of the *membrana propria* (Fig. 190). A fibrous *membrana propria* and a tunic of nonstriated muscle consisting of several circular strata complete the walls of the *vasa efferentia*.

The *vas epididymis* possesses a stratified ciliated epithelium; its convolutions are supported and held together by a loose, vascular connective tissue; toward the *vas deferens* the circular strata of muscle-fibers increase in thickness (Fig. 191).

FIG. 190.—TRANSVERSE SECTION OF AN ADULT HUMAN VAS EFFERENS TESTIS. The end of the illustration on the right is schematic. No cilia could be seen, although those of the epithelium of the epididymis were well preserved. Techn. No. 147.

The *vas deferens* consists either of a two-layered columnar epithelium or of a transitional epithelium, of a layer of connective tissue divided into a *tunica propria* and a *submucosa*, and of an inner circular and outer longitudinal stratum of smooth muscle-fibers (Fig. 192). In the initial portion of the *vas deferens* there is also a thin layer of longitudinal nonstriated muscle-fibers in the *submucosa*. The terminal portion expands forming the *ampulla*, the

FIG. 191.—TRANSVERSE SECTION OF HUMAN EPIDIDYMIS. $\times 80$. Techn. No. 147.

walls of which are thinner, but exhibit a similar structure. In the mucous membrane of the *ampulla* are branched tubular glands; the columnar cells of the epithelium contain numerous pigment-granules. The *seminal vesicles* have the same structure. The *ejaculatory duct* consists of a simple columnar epithelium and thin inner circular and outer longitudinal strata of smooth muscle-fibers.

The nerves form a plexus in the muscularis of the epididymis and of the *vas deferens*, denser in the latter, from which delicate fibers continue into the mucous membrane.

The *paradidymis* or the *organ of Giralldès*, lying between the convolutions of the epididymis, and likewise the *vas aberrans Halleri* are atrophic remains of the Wolffian body. Both consist of tubules lined by ciliated cubical epithelium and enveloped by a vascular connective tissue. The *sessile hydatid* or *hydatid of Morgagni* is a solid lobule composed of a highly-vascular connective tissue and covered by a ciliated columnar epithelium; it possesses a short pedicle, which contains a duct lined by ciliated columnar epithelium. The inconstant *stalked hydatid* is a vesicle lined by cubical epithelial cells and contains a clear fluid. The signification of the hydatids has not yet been fully explained; they are by many regarded as the remains of fetal organs,—of the proximal end of the rudimentary Müllerian duct.

FIG. 192.—TRANSVERSE SECTION OF THE INITIAL PORTION OF HUMAN VAS DEFERENS. $\times 240$ The transversely-cut longitudinal muscle-fibers of the submucosa are to be seen as minute circles and dots. Techn. No. 147.

THE PROSTATE BODY.

The prostate body consists for the lesser part of glandular tissue, for the greater part of non-striped muscle-fibers. The glandular portion is composed of thirty to fifty simple branched tubular serous glands, and is characterized by its loose structure, that is, the wide intervals between the tubules. The tubules open by two large and a number of smaller ducts into the urethra. The glandular cells are low columnar elements, which in a simple layer line the tubules. In the larger ducts the epithelium is of the transitional variety, like that in the prostatic portion of the urethra. In elderly persons the so-called *prostatic crystals*—round stratified masses of secretion up to 0.7 mm. in size—occur in the gland-tubules. The involuntary muscle-fibers, found everywhere in large quantities between the gland-lobules, are augmented toward the urethra and form a robust circular layer (the internal vesical sphincter); numerous involuntary muscle-fibers are found also on the external surface of the prostate body, where they extend to the bundles of striated muscle-fibers of the external vesical sphincter. The prostate gland and the colliculus seminalis are provided with many blood-vessels. Regarding the terminations of the nerves nothing is definitely known.

The *glands of Cowper* are compound tubular glands, whose wide tubules are clothed with a simple layer of clear columnar cells, and whose excretory duct is lined with two to three strata of cubical cells.

THE PENIS.

The penis consists of three cylindrical bodies : the two corpora cavernosa and the corpus spongiosum, which are enveloped by fascia and skin.

Each corpus cavernosum is composed of a fibrous sheath, the *tunica albuginea*, and of erectile tissue. The *tunica albuginea* is a stout connective-tissue membrane, 1 mm. thick, in which an outer longitudinal and an inner circular layer may be distinguished : the bundles are intermingled with many

FIG. 103.—FROM A TRANSVERSE SECTION OF THE CAVERNOUS PORTION OF THE HUMAN URETHRA. $\times 20$.
l, Littre's glands, the lowermost line indicates the body of the gland, the upper lines, portions of the excretory duct; *g*, blood-vessels; *m*, transverse section of longitudinally-disposed muscle-fibers; *r*, superficial cortical capillary network. Techn. No. 148.

elastic fibers. The *erectile tissue* consists of connective-tissue trabeculæ containing bundles of smooth muscle-fibers, which by means of numerous anastomoses form a network the spaces of which are lined by a single stratum of flat epithelial cells. The spaces are filled with venous blood. The thick-walled arteries pass in part into capillaries, in part open directly into the deep cortical plexus. The capillaries form a network beneath the tunica albuginea, the *superficial* (fine) *cortical plexus*, which is connected with a many-layered net of wide venous channels, the *deep* (coarse) *cortical plexus*. This lies in the superficial strata of the erectile tissue and passes gradually into the venous spaces of the latter. The so-called *helicine arteries* are small branches within slender trabeculæ, which protrude as loops in the cavernous spaces, and in an imperfect injection appear to terminate in blind ends. The veins which return the blood from the corpora cavernosa arise mostly from the deep

cortical plexus, in part out of the deeper portions of the erectile tissue. They penetrate the tunica albuginea and empty into the dorsal vein of the penis.

The *corpus spongiosum* consists of two different divisions; the central portion is a venous network formed by the conspicuously-developed veins of the submucosa of the urethra; the peripheral portion resembles in structure the corpora cavernosa, excepting that there is no direct communication of the arteries with the venous spaces. The tunica albuginea is composed of a layer of circularly-arranged bundles of fibrous tissue. The glans consists of greatly-convoluted veins, held together by a well-developed connective tissue which supports the arterioles and capillaries.

THE FEMALE REPRODUCTIVE ORGANS.

THE OVARIES.

The ovaries consist of connective tissue and glandular substance. The compact connective tissue, the *ovarian stroma*, is arranged in several strata;

FIG. 194.—TRANSVERSE SECTION OF THE OVARY OF A CHILD EIGHT YEARS OLD. $\times 10$. 1, Germinal epithelium; 2, tunica albuginea, as yet but slightly developed; 3, outermost zone of the cortex containing numerous minute follicles; 4, larger follicle; 5, inner division of cortex; 6, medulla with numerous tortuous arteries; 7, follicle cut at the periphery; 8, large follicle, the cumulus ovigerus not within the plane of the section; 9, hilus, containing wide veins. Techn. No. 149.

the outermost, the *tunica albuginea*, is composed of two or more lamellæ of variously-disposed bundles, which pass by imperceptible gradations into the stroma of the *cortex*; the latter encloses the glandular substance and is continuous with the *medulla*, which contains numerous convoluted blood-vessels and strands of smooth muscle-fibers accompanying them. The *glandular substance* is formed by a profusion of spherical epithelial sacs, the *Graafian follicles*, each of which contains an ovum. In the human ovary there are about 36,000 follicles. The majority of the follicles are microscopic in size ($4\ \mu$) and in the outermost stratum of the cortex form an arched zone embracing the entire organ except at the hilus, where the vessels and nerves enter. The larger follicles occupy

the deeper portions of the cortex. The largest, those readily perceptible by the unaided eye, when fully matured extend from the medulla to the tunica albuginea. The surface of the ovary is covered by a simple layer of very small, short cylindrical cells, the *germinal epithelium*.

Only the initial stage in the development of the ova takes place during the embryonal period; their subsequent development, from the primordial to the fully-ripened follicle, may be observed in every functionally active

FIG. 195.—FROM A VERTICAL SECTION OF AN OVARY OF AN INFANT FOUR WEEKS OLD. $\times 240$. The primordial ovum has a large nucleus with a nucleolus. The egg-tube contains three ova, surrounded by cylindrical cells. Techn. No. 149.

ovary. In the fetal period, and also after birth, there may be seen between the columnar elements of the germinal epithelium larger spherical cells with nucleolated nuclei, the *sexual cells*, specially differentiated elements of the germinal epithelium. In the course of development groups of cylindrical epithelial cells enclosing several sexual cells grow into the ovarian stroma. These groups are the *primary egg-tubes*. Each ovum becomes enveloped by a single layer of the small columnar cells and separated by constriction from the

FIG. 196.—FROM A SECTION OF THE CORTEX OF THE OVARY OF RABBIT. $\times 90$. Techn. No. 149.

remaining ova. It is now a spherical body, the *primary follicle*, which thus consists of the ovum and the epithelial cells—the so-called follicular epithelium—enclosing it. So far the developmental processes are chiefly fetal. The cells of the follicular epithelium now grow taller, multiply, and become stratified; the ovum increases in size, takes up an eccentric position within the follicle, and acquires a protecting membrane, the *zona pellucida*, which exhibits delicate radial striation and gradually augments in thickness. As the ovum

develops in size a modification of the protoplasm occurs; the greater part of it is transformed into a granular mass, the *deutoplasm*; of the original egg-

FIG. 197.—SECTION OF A LARGE GRAAFIAN FOLLICLE OF A CHILD EIGHT YEARS OLD. $\times 90$. The clear space within the follicle contains the liquor folliculi. Techn. No. 149.

FIG. 198.—AN OVUM FROM THE GRAAFIAN FOLLICLE OF THE COW. *A* magnified 50, *B* magnified 240 times. The radial striation of the zona and the perivitelline space cannot be seen.

protoplasm there remains only a small zone surrounding the eccentrically-situated nucleus and a thin stratum on the surface of the ovum. The deutoplasm and egg-protoplasm are together named *vitellus*. The nucleus is called the

germinal vesicle; it contains the *germinal spot*.* Amœboid movements have been observed in the latter. Between the vitellus and zona pellucida a narrow fissure, $1.3\ \mu$ wide, the *perivitelline space*, has been described.

The follicle develops further by continual multiplication of the cells of the follicular epithelium and a cleft appears in their midst that becomes filled with a fluid substance, the *liquor folliculi*. This liquid is in part a transudate from the blood-vessels surrounding the follicle, and is in part derived from the liquefaction of some of the cells of the follicular epithelium; it increases progressively in quantity and the follicle expands to a vesicle—the *Graafian follicle*—having a diameter of from 0.5 to 12 mm. Around the larger follicles the connective tissue of the stroma is arranged in circular lamellæ forming a sheath called the *theca folliculi*, in which an outer fibrous layer—*tunica fibrosa*—and an inner vascular layer rich in cells—*tunica propria*—may be distinguished. The stratified follicular epithelium has long been known as the *membrana granulosa*; at one point it presents a thickening, the *discus proligerus* or *cumulus ovigerus*, which encloses the ovum. The cells of the cumulus which lie next to the zona pellucida are radially placed to the ovum and form the *corona radiata* (Fig. 198). The greater part of the interior space of the follicle is occupied by the liquor folliculi.

When the Graafian follicle has attained its full development, it bursts at the pole directed toward the surface of the ovary, where its site is indicated by the attenuated and arched overlying tissue, and the ovum surrounded by the discus escapes into the pelvic cavity; the empty follicle becomes converted into the yellow body—*corpus luteum*. When fertilization does not follow the discharge of the ovum the yellow body disappears after a few weeks; it is called the *false corpus luteum*. When the escape of the ovum is followed by pregnancy, the ruptured follicle develops into the *true yellow body*, which possesses a diameter of about one centimeter and endures for years. It consists of a fibrous membrane (the former tunica fibrosa) enclosing a yellow mass formed principally by proliferation of the cells of the tunica propria, and of the metamorphosed remains of the follicular epithelium, in the center of which is a cavity filled with blood. The blood is derived from the torn vessels of the tunica propria. Later the cells become in part converted into new connective tissue, the center becomes decolored, and in place of the blood-clot a granular mass occasionally containing hematin crystals appears.

Not all the primitive follicles attain complete development. Many undergo retrogressive change. Retrograde metamorphosis of mature follicles also occurs. This process is effected as follows: first the ovum dies and then cells—in part elements of the membrana granulosa, in part leucocytes—wander into the ovum and liquefy and absorb its substance. Having completed the disintegration and resorption of the vitellus, the migrated cells perish.

The arteries of the ovary, branches of the ovarian and uterine arteries,

* The germinal spot cannot be regarded as a nucleolus, since it differs from this in its chemical relations. It is not composed of paranuclein, but of a substance resembling nuclein.

enter at the hilus, divide in the medulla, and are characterized by their tortuous course (Fig. 194). From the medulla they pass to the cortex, where they are principally distributed to the Graafian follicles in which they form capillary networks in the tunica propria. The veins form a dense plexus at the hilus of the ovary. The lymph-vessels are numerous, and may be traced to the tunica propria of the follicles. Medullated and nonmedullated nerves in large numbers enter at the hilus in company with the blood-vessels, to the walls of which the majority of them are distributed. A few of the nerves proceed to the cortex; these form a dense plexus of delicate, mostly gray fibers, which embraces the follicles and sends minute fibrils to the walls of the blood-vessels and (in the cat) between the epithelial cells of the larger follicles.

The *epoöphoron* or *parovarium* and the *paroöphoron* are embryonal remains. The former lies within the broad ligament between the ovary and oviduct and consists of a group of convoluted blind tubules, lined with ciliated columnar epithelium. The parovarium is the remains of the middle or sexual segment of the Wolffian body. The paroöphoron consists of branched tubules lined with ciliated columnar epithelium, and is embedded in the broad ligament between the ovary and uterus; it represents the posterior segment of the Wolffian body.

THE OVIDUCT.

The walls of the *oviduct* or *Fallopian tube* consist of three coats: an inner *mucons*, a middle *muscular*, and an outer *serous*. The *mucons membrane* is thrown into numerous longitudinal folds, so that on tranverse section the lumen of the narrow portion of the oviduct has a stellate outline. The folds correspond in amplitude to the size of the tube and are highest in the ampulla, where they are united to one another by minute oblique secondary plications. The thick *mucons coat* is composed of a fibro-elastic tunica propria containing numerous connective-tissue cells, and of a layer of simple ciliated columnar epithelium; the ciliary wave is directed toward the uterus. Outside of the tunica propria is an extremely thin *muscularis mucosæ* consisting of longitudinally-disposed bundles of smooth muscle-fibers. The submucosa is represented by a thin layer of fibrillar connective-tissue. The *muscular coat* consists of an inner thicker circular and an outer very thin longitudinal layer of involuntary muscle-fibers. The *serous tunic* is formed by the peritoneum and a considerable layer of loosely-united connective-tissue bundles.

The blood-vessels are especially abundant in the mucosa, where they form a narrow-meshed capillary network. The larger veins run along the bases of the longitudinal folds of the mucosa. The knowledge of the relations of the lymph-vessels and the ultimate distribution of the nerves is still imperfect.

THE UTERUS.*

The walls of the uterus, like those of the oviduct, consist of a mucosa, a muscularis, and a serosa.

* This chapter has been revised and considerably enlarged by the editor.

The *serosa* exhibits no special characteristics.

The *muscularis* consists of smooth muscle-fibers, united into bundles which interlace in all directions, so that a sharp demarcation of single layers is not possible; still, in general, three strata, more or less well-defined, may be distinguished: (1) an *inner layer* (stratum submucosum), composed chiefly of bundles disposed in a longitudinal direction; (2) a *middle layer*, the most robust, consisting of bundles having in general a circular disposition, and containing the principal ramifications of the arteries and also a well-developed venous plexus, hence the name *stratum vasculare*; (3) and an *outer layer* (stratum supravasculare), formed partly of bundles extending in a circular, partly in a longitudinal direction, the latter close beneath the serosa, with which it is intimately united. The stratification of the muscular tissue is more pronounced in the cervix, where an inner and an outer longitudinal may

FIG. 199.—FROM A TRANSVERSE SECTION OF THE MIDDLE OF THE UTERUS OF A GIRL FIFTEEN YEARS OLD. $\times 10$. a, Epithelium; b, tunica propria; c, glands; 1, inner muscular layer (stratum submucosum); 2, middle muscular layer (stratum vasculare); 3, outer muscular layer (stratum supravasculare). Techu. No. 153.

be distinguished from a middle circular layer. The volume of the muscularis is subject to great variation, dependent on the functional condition of the uterus.

The *muscle-fibers* differ somewhat from the elements of smooth muscle-tissue as found in other organs. They are elongated cells, usually spindle-shaped, or are blunted and frayed at the ends. Frequently they are forked at their extremities. Their length varies greatly; in the virgin uterus from 40 to 60 μ ; during pregnancy they increase excessively, and at the end of the same attain a size of from 300 to 600 μ . The *nucleus* (not infrequently two or more are present in one cell) is usually oval, and lies embedded in a granular substance.

The *mucosa* is sharply defined from the muscularis. It is the coat which in the different functional conditions of the uterus undergoes the profoundest, and physiologically the most important changes. A description of the histologic structure of the mucosa of the uterus can, therefore, only answer to the corresponding functional condition of the organ, and in consideration hereof will be presented in separate sections.

It is desirable to consider:—

1. The mucosa of the virgin resting organ.
2. The mucosa of the menstruating uterus.
3. The mucosa of the gravid uterus.

FIG. 200.—MUCOUS MEMBRANE OF THE RESTING UTERUS OF A YOUNG WOMAN. $\times 35$.—(After Böhm and von Davidoff.)

The mucosa of the virgin resting uterus (Fig. 200), after the advent of puberty, has a thickness of from 1 to 2 mm. and bears on its surface a single layer of ciliated columnar epithelium, $30\ \mu$ in height in the middle regions; the ciliary wave is directed toward the cervix. The tunica propria is formed of a fine fibrous tissue closely resembling embryonal connective tissue; it consists of elongated cells furnished with oval nuclei, which send out in all directions branched processes which unite with those of neighboring cells and form a cellular network, the meshes of which are occupied by lymph and numerous leucocytes.

The tunica propria supports many simple or forked gland-tubules, the upper part of which pursues a course more or less vertical to the surface of the

mucosa, while the lower half usually appears irregularly spiral. The glands extend close up to the muscularis, and here not infrequently they are bent at right angles, so that the fundus runs parallel to the muscular coat. The glands of the uterus are to be regarded as invaginations of the superficial epithelium, and consist likewise of a simple layer of ciliated epithelium resting upon a delicate basement membrane composed of anastomosing connective-tissue cells.

The blood-vessels run in a winding manner from the muscularis to the surface of the mucosa, and the *arteries*, especially, are characterized by their extremely convoluted, corkscrew-like course. At the surface they break up into capillaries and form a close network. A similar network surrounds the gland-

FIG. 201.—MUCOUS MEMBRANE OF A VIRGIN UTERUS DURING THE FIRST DAY OF MENSTRUATION. ds, Disintegrating surface; pd, pit-like depression of the mucous membrane; gl, glandular lumen very much enlarged. $\times 30$ —(Schaper.)

tubules. The *veins* proceeding from the capillaries form a plexus in the deeper strata of the mucosa, that is especially well developed in the cervix and particularly around the external orifice.

In the *cervix* the mucous membrane is thicker, and in its upper two-thirds is clothed with a single layer of tall ciliated cells (60μ high in the middle portion),* while toward the external orifice papillæ covered by a stratified squamous epithelium appear. In addition to a few scattered tubular glands, mucous follicles, the so-called *mucous crypts*, occur; they are 1 mm. wide, possess

* Transformation of these cells into goblet-cells occurs.

many evaginations, and by retention of their secretion are converted into cysts, the *ovula Nabothi*.

During the *period of menstruation* a number of progressive and retrogressive changes take place in the mucosa of the uterus, which may be grouped in three phases:—

(a) Thickening of the mucosa, accompanied by changes in its histologic structure.

(b) Menstruation proper.

(c) Regeneration.

The *initial phase* is characterized by a considerable increase in the thickness of the mucosa (up to 6 mm.), in consequence of which the surface

FIG. 202.—VERTICAL SECTION THROUGH THE MUCOUS MEMBRANE OF HUMAN UTERUS ONE MONTH PREGNANT: it shows the outlines of the glands and the division of the mucosa into an upper compact and a lower cavernous layer.—(After Minot.)

becomes irregular and the orifices of the glands open in deep depressions. The thickening of the mucosa depends in a measure on an actual increase of the tissue, produced by proliferation of the connective-tissue cells and leucocytes and by growth of the gland-tubules, which in the process take up an irregular course and become essentially wider. Simultaneously the blood-vessels, especially the veins and capillaries, undergo enormous distention, whereby the blood-supply of the organ is extraordinarily augmented. In this condition the mucosa is designated *decidua menstrualis*.

These changes are followed by a partial disintegration of the superficial strata of the mucosa, accompanied by an infiltration of blood into the sub-epithelial tissues. The molecular disintegration (associated with fatty degen-

eration) of the surface advances rapidly, the greatly-dilated superficial blood-vessels become exposed, rupture, and cause hemorrhages within the uterine cavity, which flow into the vagina and give rise to the external phenomena of *menstruation*. After this discharge of blood the mucosa becomes rapidly reduced in thickness. The surface is now entirely devoid of epithelium, and consists of connective tissue and exposed blood-vessels. This condition is immediately succeeded by the stage of *regeneration*. The hyperemia disappears rapidly, the extravasated blood is partly resorbed, partly cast off, a cellular network grows upward and restores the lost tunica propria, while from the gland-cells the epithelial covering of the mucosa is regenerated. New subepithelial capillaries are formed.

The histology of the mucosa of the uterus during *pregnancy* (*decidua graviditatis*) (Fig. 202 and Fig. 203) is, on the whole, like that of the decidua

FIG. 203.—VERTICAL SECTION THROUGH THE WALL OF A UTERUS ABOUT SEVEN MONTHS PREGNANT, WITH THE FETAL MEMBRANES IN SITU. Between the amnion and chorion are threads of the intermediate gelatinous connective tissue. $\times 30$.—(Schaper.)

menstrualis, with the alterations more pronounced. It, however, undergoes considerable modification because of its intimate relations with the developing ovum in the uterus. These relations vary, and thus in the course of development three essentially different parts of the mucosa may be distinguished:—

(a) The *decidua serotina* (*decidua basalis*), the area of the mucosa to which the ovum is attached (*placenta uterina*).

(b) The *decidua vera*, which comprises all the remaining portion of the mucosa attached to the wall of the uterus.

(c) The *decidua reflexa* (*decidua capsularis*), the portion of the mucosa which projects into the cavity of the uterus and encapsules the ovum.

The decidua serotina and vera undergo progressive development during the entire course of pregnancy and persist until its close; the decidua reflexa becomes gradually attenuated and disappears in the course of the fifth month.

A section of the greatly thickened mucosa (decidua vera and serotina) shows the same histologic details that have been described in the menstrual decidua, but with this difference, that the progressive alterations (proliferation of the connective-tissue elements, distention of the blood-vessels and glands) attain much greater proportions. A *superficial compact zone* and a *deep spongy zone* can always be distinguished (Fig. 202). The cavities in the latter are produced by the lower divisions of the gland-tubules, which have become greatly widened and very tortuous. At a later stage of pregnancy, owing to the great distention of the uterus, the lumina of the glands appear compressed and straighter (parallel to the muscular coat) (Fig. 203). Between the glands are numerous blood-vessels, spindle-cells, and multinucleated giant-cells. The epithelium of the glands begins early to loosen, and in great part the cells lie irregularly scattered in the lumen of the tubule, where they disintegrate. The

orifices of the glands are gradually obliterated, since the walls, after the loss of the epithelium, become adherent and grow together.

The *blood-vessels* of the mucosa are all dilated, especially the superficial veins and capillaries; the latter often form distended sinus-like cavities in the upper layer of the decidua. In the decidua serotina the arteries and veins open on the surface of the mucosa (Fig. 205 and Fig. 206), so that here the maternal blood circulates between the chorionic villi of the placenta (see Placenta, page 217). In the decidua vera the blood-vessels, toward the end of pregnancy, are less conspicuous.

FIG. 204.—DECIDUAL CELLS FROM THE MUCOUS MEMBRANE OF HUMAN UTERUS ABOUT SEVEN MONTHS PREGNANT. Below a "giant-cell," above to the right a cell with a karyokinetic figure. $\times 250$.—(Schäper.)

Of especial interest are peculiar, typical cells, *decidual cells*, which appear in large numbers in the mucosa of the gravid uterus. They are flattened, spherical, oval, or branched cells of conspicuous size

(0.03 to 0.1 mm.), which, in the latter half of pregnancy, assume a characteristic brown color. They possess usually but one nucleus, though occasionally two, three, or more are present, and in rare cases as many as 30 or 40.

The decidual cells are most numerous and most densely aggregated in the upper compact zone of the serotina (Fig. 203), which owes its typical character and brown color to these elements. Occasionally cells are found that are united with one another by means of protoplasmic processes. According to Minot, the decidual cells originate from connective-tissue elements, and therefore may be regarded as a modified embryonal or so-called anastomosing connective tissue.

In a cross-section of the decidua vera, in the latter half of pregnancy, it will be seen that the surface of the mucosa is covered by two distinct membranes—fetal membranes—the *chorion* and the *amnion* (Fig. 203). The chorion lies next to the decidua vera, and is intimately united with it. It consists of two layers, an epithelial and a connective-tissue layer, of which the former is

turned toward the uterine wall, the latter toward the amnion. Two similar layers may be distinguished in the amnion, but of these the epithelial layer, which consists of cubical cells, is turned toward the cavity of the uterus, while the connective-tissue stratum faces the chorion. The amnion and chorion are loosely united to each other by mucous connective tissue, in which delicate fibrils may be seen extending from one membrane to the other.

The *lymph-vessels* of the uterus form in the mucosa a wide-meshed network provided with blind branches. From this small stems proceed through the muscularis, and communicate with a close subserous network of larger channels.

The *nerves* of the uterus, medullated as well as nonmedullated, are very numerous. They branch—the medullated nerves after losing their medullary sheath—in the muscularis, and form a dense plexus in this and in the mucosa. From the latter delicate fibrils may be traced between the epithelial cells.

THE PLACENTA.*

The placenta is an organ which from a morphologic standpoint is composed of two heterogeneous parts, of which the one is produced by the mother (placenta uterina), the other by the embryo (placenta foetalis). It is the result of the intimate union of a circumscribed area of the chorion (chorion frondosum), with that portion of the mucosa of the uterus known as the decidua serotina. The placenta serves the purpose of bringing the fetal and maternal blood into the closest proximity, to render possible the interchange of materials between them. To subserve this function the organ possesses a peculiar histologic construction, in which the blood-vessels, especially in their arrangement and structure, take a prominent part.

In the histologic investigation of the placenta various obstacles are encountered, owing to its being an extremely soft spongy mass, traversed by numerous wide blood-vessels. The comprehension of the minute structure will be considerably facilitated by proceeding from the previously-mentioned fact that the finished organ is the product of two originally heterogeneous structures, the chorion on the one side, the decidua serotina on the other, and that this union is substantially effected in that the chorion, by means of numerous villous-like proliferations, penetrates the underlying serotina, the surface of which is peculiarly modified and further regressively altered for its reception, and as it were takes root in the same. For the investigation of these relations sections through the wall of the uterus with the placenta in situ, toward the end of pregnancy, are most instructive. In such a section two sharply-defined zones may be recognized: an outer compact stratum consisting of the greatly-thickened muscular coat of the uterus, covered externally by the serosa, and an inner spongy zone containing numerous inter-communicating spaces filled with blood. The latter is the placenta; that is, the united decidua serotina and chorion frondosum.

* This chapter is an entirely new addition by the editor.

FIG. 205.—SECTION THROUGH A NORMAL HUMAN PLACENTA OF ABOUT SEVEN MONTHS, IN SITU. Am. Amnion; Cho, Chorion; Vi, villus trunk; vi, sections of villi in the substance of the placenta; D, decidua basalis; Mc, muscularis; D', compact layer of decidua; Ve, uterine artery opening into the placenta. The fetal blood-vessels are drawn black; the maternal blood-spaces are left white; the chorionic tissue is stippled except the canalized fibrin, which is shaded by lines; the remnants of the gland cavities in D'' are stippled dark.—(After Minot.)

The accompanying illustration (Fig. 205) shows their relations under low magnification, which will be elucidated by referring to the schematic representation in Fig. 206.

The surface of the placenta directed toward the cavity of the uterus is covered by a compact stratum, the *membrana chorii*, which is composed chiefly of fibrillar connective tissue, and in which the main branches of the umbilical blood-vessels run. The outer surface of the chorion is covered by a delicate membrane, the placental portion of the amnion, which, as previously stated, consists of an inner epithelial and a connective-tissue layer, and is connected with the chorion by means of embryonic connective tissue. The other surface of the *membrana chorii*, that directed toward the wall of the uterus, is closely beset with innumerable villous like structures, large and small, which in the

FIG. 206.—DIAGRAM OF HUMAN PLACENTA AT THE CLOSE OF PREGNANCY. Comp. with Fig. 205.—(Schaper.)

upper part of the placenta form a dense tangle, and whose terminal ramifications are embedded in the cleft, uneven substance of the serotina. On closer study of this villous tangle it will be seen that the larger stems run a more or less direct course from the chorion to the serotina, in order to secure a firm union with the latter, while their many much-branched lateral twigs usually establish no connection with the uterine portion of the placenta, but terminate free in the blood-spaces, the so-called *intervillous spaces*, between the chorion and serotina. Dependent upon these relations the branches of the chorionic villi are divided into "*roots of attachment*" or *main stems*, and *free processes* or *floating villi*. From the chorion a branch of the umbilical artery enters each main stalk and, within the terminal ramifications of the villus, breaks up into a dense capillary network from which the umbilical veins take their origin and carry back the blood from the chorion through the umbilical cord to the

fetus. Accordingly, the blood-vessel system of the fetal placenta is entirely closed. Nowhere does a direct intermingling of maternal and fetal blood occur.

A cross-section of one of the smaller chorionic villi, highly magnified, shows that it is chiefly composed of mesenchymal tissue (mucous tissue), in which the blood-vessels are embedded (Fig. 207). This central supporting substance is covered by an irregular and not everywhere continuous stratum of epithelium. In the earlier months of development two distinct strata may be distinguished in the epithelium of the villi: an inner, lying immediately upon the supporting tissue, in which the cells possess large nuclei and definite contours, so that in the main they are distinctly separated from one another; and an outer layer, consisting of a continuous protoplasmic mass—*syncytium*—containing numerous small irregularly-scattered nuclei. Toward the end of pregnancy, however, the epithelium of the villi undergoes great alteration, as appears in the

FIG. 207.—CROSS-SECTION THROUGH A SMALLER (A) AND LARGER (B) CHORIONIC VILLUS OF HUMAN PLACENTA AT THE END OF PREGNANCY. $\times 250$.—(Schäper.)

illustration (Fig. 207). On the larger villi a true epithelial investment has almost entirely disappeared and instead, isolated accumulations of large round nuclei are found; they stain intensely, are embedded in a clear, homogeneous substance, and form protuberances (*Zellknoten*, *cell-patches*) on the surface of the villi. Between these cell-patches the connective tissue of the villi is frequently covered only by a thin, homogeneous stratum, or in other cases (especially in smaller villi) this stratum still retains more or less the character of the protoplasm containing scattered nuclei. There are many indications that the latter is the remains of the syncytium, while the cell-patches probably originated in the primitive inner stratum of the epithelium of the villi. In many places the syncytium becomes transformed into a peculiar hyaline substance, permeated by fissures, which often lies upon the chorion in dense strata, and is called *canalized fibrin*.*

* It has not been as yet determined with certainty whether the epithelium of the villi of the human placenta is derived entirely from the epithelium of the chorion, or whether the epithe-

The histologic structure of the maternal portion of the placenta—*placenta uterina*—in its essential features has been described in connection with the decidua in the preceding chapter. Certain peculiarities, however, as well as the union of the maternal and fetal placenta in a functional whole require a brief consideration.

The placental portion of the decidua (Fig. 205), that forming the lower stratum of the placenta (basal-plate), becomes greatly thinned (0.5 to 1.0 mm.), however, as in the extraplacental portion, an upper compact layer, and a lower cavernous layer (gland lumina) may be distinguished. The decidual cells are extremely numerous and lie closely crowded. A honeycombed structure of connective-tissue septa (*septa placenta*) arises from the surface of the serotina, directed toward the intervillous spaces, and penetrates between the villi of the chorion, separating the latter into lobes or cotyledons. Only in the peripheral regions of the placenta do these septa reach to the membrana chorii, where frequently they form on the inferior surface of the latter a thin membranous stratum, the *decidua placentalis subchorialis*. On the margin of the placenta the serotina gradually increases in thickness and passes into the vera, at which point it is closely applied to, and firmly united with, the chorion. Within the area of the placenta, however, the chorion and serotina are far apart, and the space between them is filled with the above-described chorial villi and the blood circulating between them; it is *maternal* blood that surrounds the villi on all sides, and is thus brought into the closest relation with the fetal circulation.

Of especial interest is the behavior of the blood-vessels within the placenta uterina (Fig. 205 and Fig. 206). Numerous *arteries* from the muscularis of the uterus penetrate the serotina, in which they make cork-screw-like tours, in the course of which they lose their muscular coat and continue as wide tubes consisting alone of the lining endothelium. Near the surface of the decidua they usually bend sharply at right angles, and then open directly into the intervillous spaces of the placenta.* *Nowhere do the arteries break up into capillaries.* The *veins* (like-

lium of the serotina participates in its composition. Recent investigations, however, as well as comparative anatomical facts, indicate that only the inner epithelial stratum of the villi comes from the chorionic epithelium, while the syncytium is derived directly from the mucosa of the uterus, the epithelium of which, on the ingrowth of the chorial villi, becomes closely applied to, and blends with, the epithelium of the latter.

* In regard to the relation of the decidual blood-vessels to the intervillous spaces there are two conflicting theories. According to the one, the intervillous spaces are independent clefts, without proper walls, that are formed in the course of development between the fetal and maternal portions of the placenta, and with which the blood-vessels opening on the surface of the decidua are in direct communication. Accordingly the villi of the chorion are in direct contact with the maternal blood circulating in these spaces. The opposite view regards the blood-spaces of the placenta as the enormously widened capillaries of the decidua, which, during the mutual process of intergrowth between the placenta uterina and placenta fetalis, the developing villi of the chorion have invaginated. According to this the blood-vessel system of the decidua is closed and the arteries and veins communicate through a system of capillary lacunae (the intervillous spaces). Further, the chorial villi are not directly bathed in the maternal blood, but

wise endothelial tubes, though wider than the arteries) also are in direct communication with the placental spaces; they enter the decidua usually under a very narrow angle, run more or less parallel to the surface, and unite in the deeper strata in a wide venous plexus. In accordance with the description of these conditions of the vessels, the arteries and veins within the serotina can no longer be recognized by the histologic structure of their walls, but can only be distinguished by their width and their course. The arteries are, in addition, usually characterized by a thin homogeneous enveloping stratum that stains intensely with carmine, and in which a few scattered nuclei are found. This peculiar layer is probably a product of the degenerated muscular coat.

THE VAGINA AND THE GENITALIA.

The *vagina* is formed by a mucous membrane, a muscular tunic, and a fibrous coat.

The *mucous membrane* is composed of a stratified scaly epithelium and a tunica propria beset with papillæ; the latter is built up of small interlacing bundles of connective tissue profusely intermingled with elastic fibers, and contains a variable quantity of leucocytes. The latter occasionally exist in the form of solitary nodules; in this case, in these localities numerous wandering leucocytes are found in the epithelium. The mucosa rests on a *submucosa* consisting of loosely-united connective-tissue bundles and of robust elastic fibers. Glands are absent within the vaginal mucous membrane.

The *muscular coat* comprises an inner circular and an outer longitudinal layer of smooth muscle-fibers.

The outer *fibrous tunic* is a dense connective-tissue structure, rich in elastic fibers.

The blood- and lymph-vessels are arranged in parallel horizontal networks in the submucosa and the tunica propria. Between the bundles of the muscular tunic lies a close network of wide venous channels. The nerves form a plexus beset with many small ganglia in the outer fibrous tunic.

The mucous membrane of the *external genitalia* in the vicinity of the clitoris and the urethral orifice differs from the vaginal mucosa in the possession of numerous mucous glands, 0.5 to 3 mm. in size, and on the labia minora in the presence of sebaceous follicles (without hair-follicles) 0.2 to 2 mm. in size. The clitoris repeats on a diminutive scale the structure of the penis; end-bulbs and tactile-corpuscles occur in the glans.

The *glands of Bartholin* are the homologues of the glands of Cowper in the male.

are separated from it by a thin stratum of cells, the capillary endothelium, which lies directly upon them. Recent investigations of Keibel apparently support the latter view, since in a human placenta in an early stage of development he succeeded in tracing the endothelium of the decidual blood-vessels into the intervillous spaces, and demonstrating it as a continuous stratum on the surface of the chorionic villi. It is possible that in the further development of the placenta this endothelial covering undergoes regressive change, so that in later stages it cannot, as a rule, be demonstrated.

The labia majora are folds of the integument and possess the same structure.

The acid vaginal secretion contains desquamated scaly epithelial cells and leucocytes, and not infrequently an infusorium, trichomonas vaginalis.

IX. THE SKIN AND ITS APPENDAGES.

The skin is composed principally of connective tissue, which however is nowhere exposed, but is protected by the superficial epithelium with which it is connected. The connective-tissue portion of the skin is called the *corium*, *derma*, or *true skin*; the epithelial portion, the *epidermis* or *cuticle*. The appendages of the skin, the *nails*, and the *hairs*, as well as the *glands* and the *hair-follicles* embedded within the corium, are products of the epidermis.

THE SKIN.

The surface of the *corium* is marked by many furrows, which intersect and enclose rectangular or lozenge-shaped areas or run parallel between minute ridges. The lozenge-shaped areas may be seen on the surface of the greater part of the body, while the ridges are confined to the volar surface of the hand and the plantar aspect of the foot. These areas and ridges are beset with numerous conical elevations, the papillæ, whose number and size vary greatly in different regions. They are largest (up to 0.2 mm. high) and most numerous on the palm of the hand and on the sole of the foot; they are very slightly developed in the skin of the face.

The corium is composed chiefly of interlacing fibrous connective-tissue bundles, mingled with elastic fibers, cellular elements, and smooth muscle-fibers. In the superficial strata of the corium the fibrous bundles are delicate and are united in a dense feltwork; in the deeper strata they are larger and form a coarse-meshed network. These differences have led to the recognition of two strata in the corium: a superficial stratum beset with papillæ, *stratum papillare*, and a deep stratum, *stratum reticulare*. There is no sharp demarcation between the two strata, the one gradually blending with the other (Fig. 208). The stratum reticulare is connected with an underlying network of loosely-united bundles of fibrous tissue, the wide meshes of which contain clusters of fat-cells; this is the *stratum subcutaneum*. Much adipose tissue in the interfascicular spaces of this stratum leads to the formation of the *panniculus adiposus*. The tissue of the subcutaneous stratum is firmly or loosely united with the fibrous sheaths of the muscles or with the periosteum of the bones. The elastic fibers, which are thin in the stratum papillare and stout in the stratum reticulare, form networks distributed uniformly throughout the corium. The cells include spindle-shaped and plate-like elements, leucocytes and fat-cells. The number of the cellular elements is extremely variable.

The muscle-fibers belong almost exclusively to the non-striped variety, and the majority are attached to the hair-follicles; only in a few situations are they disposed as a membrane (tunica dartos, nipple). Striated muscle-fibers occur in the skin of the face, where they radiate from the mimetic muscles.

The *epidermis* consists of a stratified squamous epithelium, in which at least two sharply-defined zones may be distinguished: a deep, soft, so-called mucous zone, *rete mucosum* (stratum Malpighii), which fills the depressions between the papillæ of the corium, and a superficial firm zone, the *stratum corneum*. Both strata are composed exclusively of epithelial cells, which vary in appearance in the different layers. In the deepest layer of the rete mucosum the cells are cylindrical and possess oval nuclei; these are followed by several layers of polyhedral cells beset with numerous minute spines—prickle-

FIG. 208.—VERTICAL SECTION OF THE SKIN OF FINGER OF ADULT MAN. $\times 25$. With this magnification the stratum granulosum is not visible. Techn. No. 154

cells. The spines are delicate thread-like processes, which penetrate the intercellular cement-substance and unite neighboring cells to one another. In the rete mucosum new cells are continually being formed by indirect division.

The *stratum corneum* is not everywhere of the same structure. In localities where the epidermis is well developed, as on the palm of the hand and the sole of the foot, several layers of cells characterized by highly-refracting granules—*granules of eleidin*—lie at the inner border next to the rete mucosum. The granules of eleidin, or keratohyaline granules, are produced by the cornification of parts of the cell-protoplasm.* These layers form the *stratum granulosum*. In the next layer the granules dissolve and blend with the protoplasm

* These granules dissolve in a solution of potassium hydroxid and, therefore, are not composed of keratin, which is insoluble in this reagent.

not yet transformed into horny substance, and form a uniformly clear zone, the *stratum lucidum*. This is covered by the deep *stratum corneum* proper. In this stratum all the non-cornified parts of the cell, under the influence of the atmosphere, become desiccated; and so it happens that each cell contains a delicate horny mesh-work, and—as the intercellular bridges also become cornified—is enveloped in a horny membrane. The nucleus desiccates; the space which it occupied persists for a long period. The partly cornified, partly desiccated cells are only slightly flattened.

In situations where the epidermis is thinner, the stratum granulosum is narrow and interrupted. The stratum lucidum is wanting. The horny cells of the stratum corneum become extremely compressed and united in lamellæ. The last trace of the nucleus disappears.

FIG. 209.—FROM A SECTION THROUGH THE SKIN OF THE SOLE OF THE FOOT OF ADULT MAN. No. 360. Techn. No. 154.

The surface of the horny stratum undergoes a continual physiologic desquamation; the resulting loss is compensated by the pushing upward of the growing elements of the rete mucosum.

The color of the skin is due to the deposition of fine granules of pigment between and within the cells of the deeper layers of the epidermis; only in certain localities, for example, in the vicinity of the anus, are pigmented connective-tissue cells found in the adjacent corium.

With regard to the source of the pigment there are two theories, of which the one attributes its production to the connective tissue, the other to the epi-

thelium. According to the first, the so-called "transportation" theory, the pigment is carried to the epithelium by pigmented connective-tissue cells that wander from the corium into the epidermis, and then disintegrate. In the human hair-bulb pigmented forms presenting great diversity in outline are found between the epithelial elements; some of these figures are cells, but it has not been demonstrated with certainty that they are connective-tissue cells, others are not cells, but intercellular clefts filled with pigment. The second theory is supported by the developmental history, which teaches that the pigment originates in the epithelium without the intervention of connective-tissue cells. The pigment of the retina also is certainly and exclusively of epithelial origin.

THE NAILS.

The nails are horny laminae which rest upon the *nail-bed*, a special modification of the skin. The nail-bed is bounded on the sides by the *nail-walls*, a pair of sloping folds with the descent forward. The nail-bed and nail-wall embrace a furrow, the *nail-groove*, in which the lateral borders of the nail are

FIG. 210.—DORSAL HALF OF A CROSS-SECTION OF THE THIRD PHALANX OF CHILD. $\times 15$. The ridges of the nail-bed appear in cross-section like papillae. Techn. No. 155.

inserted. The posterior border of the nail, the *nail-root*, rests in a similar but deeper groove, the *matrix*, in which the principal growth of the nail takes place. (Some authors name the whole nail-bed matrix, which is in a measure justified by the growth of the nail in thickness which occurs here.)

The anterior free edge of the nail projects over the *nail-ridge*, a small seam-like prominence at the distal end of the nail-bed.

The *nail-bed* consists of corium and of epidermis. The fibro-elastic bundles of the corium are in part disposed parallel to the long axis of the fingers, in part vertically from the periosteum of the phalanx to the surface. There are no papillae on the corium, but minute longitudinal ridges. They begin low at the matrix, increase in height toward the front of the nail, and terminate abruptly at the point where the latter leaves its bed. The epithelium is of the stratified scaly variety, of the same structure as that of the rete mucosum of the epidermis. It covers the ridges of the nail-bed, fills up the furrows between them, and is sharply defined from the substance of the nail. The matrix, likewise, consists of corium and epidermis; the corium is distinguished

by its tall papillæ, the stratified scaly epithelium is very thick and is not sharply defined from the nail-substance, but passes gradually into the latter. This is the place where by continual division of the epithelial cells the material for the growth of the nail is furnished. The extent of the matrix is indicated by the *lunula*, a white convex area, visible to the unaided eye, produced by the thick, uniform rete mucosum. The *nail-wall* and the *nail-fold* (the margin of the groove overhanging the root of the nail) exhibit the same general structure as the skin; the rete mucosum blends gradually with the epithelium of the nail-bed, while the horny stratum extends into the nail-groove and as "eponychium" covers a small portion of the edge of the nail, but soon diminishes in thickness and disappears (Fig. 210).

The *nail* itself consists of horny epithelial scales, very firmly united with one another, which possess a nucleus and differ in this respect from the horny cells of the stratum corneum of the epidermis (Fig. 211).

THE HAIR.

The hairs are flexible, elastic horny threads, which are distributed over nearly the entire surface of the body and on the integument of the cranium are united in small groups. The part of the hair which projects beyond the free surface of the skin is called the *shaft*; the portion obliquely embedded within the integument, the *root*; at its lower extremity the latter terminates in a bulbous expansion, the *hair-bulb*, which embraces a formation of the corium, the *hair-papilla* (Fig. 212).

Each hair-root is inserted in the *hair-follicle*, a modification of the skin in the formation of which both corium and epidermis participate; the parts furnished by the latter are the *epithelial root-sheaths*, the portion originating from the corium is the *dermal or fibrous sheath*. Into the follicle, laterally, two to five glands open, the *sebaceous glands*. Bundles of smooth muscle-fibers, the *arrectores pilorum*, pass obliquely from the upper surface of the corium and attach themselves to the fibrous sheath of the hair-follicle, beneath the sebaceous glands; the point of insertion of these fibers is always on the side toward which the hair inclines; when they contract, the follicle and the shaft become erect.

The hair consists entirely of epithelial cells, arranged in three well-defined strata: the *cuticle*, which covers the surface; the *cortical substance*, which contributes the chief bulk; the *medulla*, which occupies the axis of the hair.

The *cuticle* consists of a single layer of transparent imbricated scales—horny epithelial cells without nuclei.

The *cortical substance* of the shaft consists of elongated horny epithelial cells with attenuated nuclei, which are intimately united with one another; on the root the cells become softer and rounder, their nucleus correspondingly more spherical, as they approach the hair-bulb.

FIG. 211.—ELEMENTS OF HUMAN NAIL. $\times 240$. Techn. No. 156.

The *medulla* is absent in many hairs ; when it is present it does not extend through the entire length of the hair. It consists of cubical, finely-granular epithelial cells, which contain a rudimentary nucleus and are usually disposed in twofold rows.

The colored hairs contain pigment, diffused and in the form of granules, which occurs in part between and in part within the cells of the cortical substance. In every hair which has attained its full development *minute air-vesicles* occur ; they are found in the cortical substance as well as in the medulla, and also in the intercellular clefts.

The *follicle* of fine (lanugo) hairs is formed alone by the epidermal root-sheaths, but in coarser hairs the corium participates in its construction. In the follicles of the latter the following strata may be distinguished : an *outer longi-*

FIG. 212.—FROM A THICK CROSS-SECTION OF HUMAN SCALP. $\times 20$. Techn. No. 160.

tudinal stratum formed of loosely-united, longitudinally-disposed bundles of white fibrous tissue, mingled with elastic fibers and richly supplied with blood-vessels and nerves ; a *middle circular stratum*, thicker, and consisting of small fibrous bundles circularly arranged ; and an *inner* clear, homogeneous, narrow belt, the *glassy* or *hyaline membrane*, resembling in character the elastic membranes. Elastic fibers do not occur in the middle layer nor in the papilla. These three strata are derived from the corium and together constitute the *dermal* or *fibrous sheath* of the follicle. Within the hyaline membrane lies the *outer root-sheath* ; it is a continuation of the rete mucosum of the epidermis and consists of stratified scaly epithelium ; inward to this lie continuations of the stratum corneum and stratum granulosum, which extend to the point where the ducts of the sebaceous glands open into the follicle. Immediately below

(toward the papilla) the *inner root-sheath* begins abruptly, which in the lower portion of the follicle is differentiated into two sharply-defined layers; the

FIG. 213.—ELEMENTS OF HUMAN HAIR AND HAIR-FOLLICLE. $\times 240$. 1. White hair; 2. scales of the cuticle; 3. cells of the cortical substance of the shaft; 4. cells of Huxley's layer; 5. cells of Henle's layer, having the appearance of a fenestrated membrane, 6. cells of the cortical substance of the root. Techn. No. 159.

FIG. 214.—FROM A HORIZONTAL SECTION OF HUMAN SCALP. $\times 240$. Cross-section of a hair and hair-follicle from the lower half of the root. Techn. No. 162.

outer, *Henle's layer*, consists of a single or double row of epithelial cells without nuclei, while the inner, *Huxley's layer*, is formed of a simple stratum

of nucleated cells. The inner surface of this layer is lined by a delicate membrane, the *cuticle of the root-sheath*, which exhibits the same structure as the cuticle of the hair. Toward the base of the follicle the outer root-sheath diminishes in thickness and disappears; the strata of the inner root-sheath lose their sharp demarcation and are gradually transformed into the spherical cells of the hair-bulb.

DEVELOPMENT OF THE HAIR.

The first anlage of the hair and the hair-follicle appears at the end of the third embryonal month in the form of a local thickening of the epidermis, which is effected chiefly by a proliferation of the cells of the deepest layer of the rete mucosum. This thickening grows in length within the corium and forms a solid epidermal peg—the *hair-germ*—which terminates in an expanded,

FIG. 215.—FROM A VERTICAL SECTION (A) OF THE SKIN OF THE CHEEK OF A FOUR MONTHS' HUMAN EMBRYO AND (B, C, D) OF THE SKIN OF THE FOREHEAD OF A FIVE MONTHS' HUMAN EMBRYO. $\times 80$. E, Epidermis, consisting throughout of nucleated epithelial cells; C, corium; s, thickening; hk, hair-germ; hk, connective-tissue hair-follicle; p, papilla; aww, outer root-sheath; s, axial portion, in which in the upper division the separation into (rw) an inner root-sheath, and (h) the hair is visible; t, anlage of the sebaceous glands. Techn. No. 161.

club-shaped extremity. Meanwhile the papilla and the dermal portion of the hair-follicle develop by differentiation of the connective tissue of the surrounding corium. The hair-germ separates into an outer stratum and an inner axial cord. The former becomes the outer root-sheath; the peripheral portion of the axial strand becomes the inner root-sheath; the central part, the hair. The sebaceous glands arise as local outgrowths of the outer root-sheath (Fig. 215).

The development of hairs in the manner described may occur after birth and until late in life.

GROWTH AND SHEDDING OF THE HAIR.

The growth of the hair is effected by continual mitotic division of the epithelial elements around the papilla, and by the transformation of the new cells into horny elements which annex themselves from below to previously

cornified cells. Thus, the tip is the oldest, the portion lying immediately above the hair-bulb the youngest part of the hair.

At birth all the hairs are shed and replaced by others. In the adult replacement of the dead hairs of the scalp and beard occurs continually, but not periodically. (With regard to the shedding of the other hairs nothing is definitely known.)

The minute details of the process are as follows: the hair-bulb becomes horny and frayed like a brush; the now dead hair is separated from the papilla and pushed upward by the pressure from below of the young elements produced by the division of the cells around the papilla; the empty root-sheaths collapse; at their inferior extremity lies the hair-papilla atrophied and altered in form

FIG. 216.—FROM A VERTICAL SECTION OF THE HAIRY SCALE OF ADULT MAN. $\times 40$. Techn. No, 162.

(Fig. 216). After a (often long) period the epithelial elements of the empty root-sheaths begin to grow and form a new hair-germ, from which the new hair develops by the same processes as the embryonal hair. The new hair pushes upward under the effete hair, which after a shorter or longer period falls out.

THE GLANDS OF THE SKIN.

The sebaceous glands are either unbranched or branched simple saccular glands. Each gland consists of a short excretory duct and of a variable number of acini. The duct is lined by stratified scaly epithelium, an extension of the outer root-sheath, which undergoes a gradual decrease in the number of its layers and passes into the epithelial lining of the acini. This consists at first

of low cuboidal cells, that are followed by spherical or polyhedral elements, varying in size, which fill the gland-sac and exhibit all the transitional phases in the process by which the cell is converted into the secretory product of the gland. The secretion, the *sebum*, during life is a semifluid substance consisting of fat and disintegrated cells. The sebaceous glands occur as the appendages of the hair-follicles of the larger hairs, but in the case of the lanugo hairs these relations are apparently reversed and the follicles of the latter appear as the appendages of the powerfully-developed sebaceous glands (Fig. 217 *A*). The sebaceous glands are distributed with the hair over the entire body, and are wanting only where the former are absent—on the palm of the hand and on the sole of the foot. There are, however, sebaceous glands that are not associated with hair-follicles; for example, on the red edge of the lips, on the labia minora, on the glans, on the prepuce of the penis; in the latter situation they are known as Tyson's glands. The sebaceous glands are always situated in the

FIG. 217.—*A*. FROM A VERTICAL SECTION THROUGH THE ALA NASI OF CHILD. $\times 40$. *C*, Stratum corneum; *M*, rete mucosum; *d*, sebaceous gland consisting of four follicles; *a*, duct of the same; *n*, lanugo hair, about to be shed; *h*, hair-follicle of the same, at the base of which a new hair, *x*, is forming. *B*. FROM A VERTICAL SECTION OF THE SKIN OF THE ALA NASI OF AN INFANT. $\times 240$. Follicles of a sebaceous gland containing gland-cells in various stages of secretion. Techn. No. 162.

stratum papillare of the corium. Their size varies from 0.2 to 2.2 mm.; the larger are found in the integument of the nose, where their excretory ducts are visible to the unaided eye.

The *coil-glands* (sudoriparous or sweat-glands) are long, unbranched tubules, whose lower ends terminate in a greatly convoluted spherical mass, having a diameter of 0.3 to 7 mm. (of the latter size in the axilla). Two parts are distinguished, the *excretory duct* and the *coil*. The former runs a straight or a sinuous course through the corium, enters the epidermis between two papillæ, passes in a spiral through the stratum corneum, and opens on the surface of the skin by a rounded orifice, the sweat-pore, just visible to the naked eye. The walls of the duct consist of longitudinally-disposed bundles of fibrous connective tissue, lined within by several layers of cubical epithelial cells. The *coil* is a greatly convoluted simple canal, the walls of which consist of a

simple layer of cubical cells, containing granules of pigment and of fat, surrounded by a delicate membrana propria. In well-developed glands longitudinally-disposed smooth muscle-fibers occur between the membrana propria and the gland-cells (Fig. 208). Branched tubules have been observed only in the axillary and circumanal glands.

The secretion is usually an oily fluid substance, for the purpose of lubricating the skin; only under the influence of altered innervation do the coil-glands discharge the watery liquid called sweat. The coil-glands are distributed over the entire surface of the skin and are absent only on the glans and

FIG. 218.—FROM A VERTICAL SECTION OF THE SKIN OF THE SOLE OF HUMAN FOOT. $\times 50$. *sc*, Stratum corneum; *sg*, rete mucosum; *a*, artery; *v*, vein; *a' v'*, branches to panniculus adiposus; *a'' v''*, branches to coil-glands; *k*, duct of the same, *vx*, vein accompanying this. Techn. No. 164.

on the inner surface of the prepuce. They are most numerous in the skin of the palm of the hand and of the plantar surface of the foot.

THE BLOOD-VESSELS, LYMPH-VESSELS, AND NERVES OF THE SKIN.

The *arteries* originate in a network lying over the fasciæ and pass upward to the surface of the skin; in their course they form three separate capillary networks lying in different planes. The deepest supplies the adipose tissue of the subcutaneous stratum; that occupying the next level appears in the form

within and between the gland-lobules. Lymphatic networks also occur in the vicinity of the ampullæ and the areolæ.

The nerves, as in other glands, do not form a direct connection with the secreting cells, but probably are all distributed to the blood-vessels.

Milk microscopically consists of a clear fluid, the *milk plasma*, in which *oil-globules*, 2 to 5 μ in size, are suspended. Owing to the fact that the globules do not coalesce, the presence of a delicate membrane of casein is assumed. In addition, isolated cells enclosing oil-globules (leucocytes?) are found in the milk.

The elements of the milk secreted before and in the first few days after parturition include, beside the oil-globules, the so-called colostrum-corpuscles, nucleated cells, some of which contain minute yellow-colored and larger uncolored fat-droplets, others only uncolored fat-droplets.

The mode in which the glandular epithelium participates in the formation of the milk-globules and the colostrum-corpuscles is not yet altogether clear. Only this much is known with certainty, that the cells do not perish in the act of secretion. It is a question whether the fat within the glandular cells is discharged alone or with the portion of the cell directed toward the lumen of the acinus.

X. THE EYE AND ITS APPENDAGES.

The organ of vision consists of the eyeball, the optic nerve, the eyelids, and the lacrymal glands.

THE EYEBALL.

The eyeball (*bulbus oculi*) is a hollow globe, which encloses formed and fluid contents. The walls of the eyeball are composed of three coats: (1) the *tunica externa*, a fibrous membrane in which an anterior transparent division, the *cornea*, may be distinguished from the remaining opaque portion, the *sclera*; (2) the *tunica media*, rich in blood-vessels, which includes three divisions,—the *choroid*, the *ciliary body*, and the *iris*; (3) the *tunica interna*, the *retina*, which contains the specialized terminal apparatus of the optic nerve. The formed contents within the eyeball are the *lens* and the *vitreous body*.

THE TUNICA EXTERNA.

The *cornea* consists of five strata, which enumerated from before backward are the following:—

1. The anterior epithelium.
2. The anterior basal membrane.
3. The substance proper.
4. The posterior basal membrane.
5. The posterior endothelium.

The *anterior epithelium* is a stratified scaly epithelium consisting of a lowermost layer of sharply-contoured columnar cells, which is followed by three or four (more in animals) layers of polyhedral cells, that in turn are covered by several strata of flattened elements still possessing nuclei. The thickness of the epithelium in man is 0.03 mm. At the rim of the cornea the epithelium is continuous with that of the sclera.

The *anterior basal membrane* (Bowman's membrane, lamina elastica anterior) in man is a conspicuous stratum, about 0.01 mm. thick, and almost homogeneous in appearance. The surface is provided with minute serrations and ridges for attachment to the columnar cells of the anterior epithelium. Posteriorly it passes gradually into the substantia propria of the cornea, of which it is a special modification.

FIG. 222.—VERTICAL SECTION OF HUMAN CORNEA. $\times 100$. Techn. No. 169 b.

The *substance proper* constitutes the chief bulk of the cornea. It consists of delicate parallel fibrillæ, which are united by an interfibrillar cement-substance into bundles of nearly uniform thickness; the bundles in turn are united by an interfascicular cement-substance into flat lamellæ, which lie in many superposed strata and are held together by an interlamellar cement-substance. The lamellæ are arranged parallel to the surface of the cornea and run in meridional curves one above the other, so that a vertical section through the center of the cornea shows alternate longitudinal and transverse bundles. A number of bundles running obliquely, the so-called arcuate fibers, unite each lamella with its neighbor above or below; especially well-developed arcuate fibers occur in the anterior strata of the substantia propria.

Embedded in the cement-substance is an intercommunicating system of branched canaliculi, the *lymph-canaliculi*, which at many places are expanded to broad oval lacunæ, the *corneal spaces*. The latter lie between the lamellæ, while the canaliculi also penetrate between the bundles. The lacunæ and canaliculi contain a serous fluid and cells,—“fixed” *corneal corpuscles* and

within and between the gland-lobules. Lymphatic networks also occur in the vicinity of the ampullæ and the areolæ.

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wandering cells. The corneal corpuscles are flattened connective-tissue cells possessing large nuclei; they lie against one wall of the lacunæ (Fig. 224).

The *posterior basal membrane* (membrane of Descemet, lamina elastica posterior) is a clear, glassy elastic layer, 0.006 mm. thick. In adult man the posterior surface, at the periphery of the cornea, is beset with hemispherical protuberances.

FIG. 223.—HORIZONTAL SECTION OF CORNEA OF OX. Silver-preparation; negative picture; the canallicular system is light upon a dark ground. $\times$ about 240. Techn. No. 173.

FIG. 224.—HORIZONTAL SECTION OF CORNEA OF RABBIT. Positive picture. $\times$ about 240. Techn. No. 174.

FIG. 225.—VERTICAL SECTION THROUGH A PART OF THE HUMAN SCLERA AND THE ENTIRE CHOROID. $\times$ 100. *g.* Larger vessels; *p.* pigment cells; *c.* cross-sections of capillaries. Techn. No. 169 *c.*

The *posterior endothelium* is composed of a single layer of flat polygonal cells with slightly projecting nuclei.

The *sclera* consists principally of interlacing bundles of fibrous connective tissue, extending for the most part in meridional and equatorial directions. In

addition, delicate elastic fibers arranged in networks and flattened connective-tissue cells are present; the latter, like the corneal corpuscles, lie in lacunæ, which differ from the corneal spaces only in having more irregular outlines. Between the sclera and the choroid is a layer of loose, highly-elastic tissue containing branched pigmented cells and flattened elements free from pigment ("endothelial" cells). On separating the two coats a portion of this tissue adheres to each; that on the sclera is called the *lamina fusca scleræ*, that on the choroid, *lamina suprachorioidea*.

The sclera is thickest posteriorly (1 mm.), and becomes gradually thinner toward the cornea.

THE TUNICA MEDIA.

The *choroid* is characterized by the great abundance of its blood-vessels, which are arranged in two layers. The superficial layer, adjoining the lamina suprachorioidea, the *layer of large blood-vessels*, comprises the ramifications of

FIG. 226.—A. FROM A TEASED PREPARATION OF HUMAN CHOROID. $\times 240$. *p*. Pigment cells; *e*, elastic fibers; *k*, nucleus of a flat nonpigmented cell; the cell body is not visible.
B. PORTION OF HUMAN CHORIOCAPILLARIS AND THE ADHERENT HYALOID MEMBRANE. $\times 240$. *c*. Wide capillaries, some of which contain (*b*) blood-corpuscles; *e*, hyaline membrane, showing a fine "lattice-work." Techn. No. 170 a.

the arterial and venous channels, which are embedded in a supporting tissue called the *stroma*, consisting of networks of fine elastic fibers and numerous branched pigment-cells. In addition, the stroma contains the tissues accompanying the large arteries; namely, fibrillar connective tissue, smooth muscle-fibers, and nonpigmented plate-like cells united in delicate endothelial membranes. The deeper layer, the *membrana choriocapillaris*, or layer of capillary networks, is composed of a narrow-meshed net of capillaries, between which no formed elements are found. Between the two layers of blood-vessels lies the *boundary zone*, a portion of the stroma consisting of networks of fine elastic fibers and almost devoid of pigment. In ruminants and horses this zone consists of wavy bundles of connective tissue, to which is due the metallic reflex seen in the eyes of these animals. This shining membrane is known as the *tapetum fibrosum*. The similar iridescent *tapetum cellulosum* of carnivora is composed of several strata of plate-like cells containing numerous minute crystals.

Attached to the membrana choriocapillaris is the *glassy membrane* or *vitreous lamina*, a structureless lamella, about 2μ thick, possessing delicate lattice-like markings on its outer surface. The polygonal areas observed on its

FIG. 227.—MERIDIONAL SECTION THROUGH THE RIGHT IRIDO-CORNEAL ANGLE OF MAN. $\times 30$. 1. Epithelium. 2. Connective-tissue of the conjunctiva. 3. Sclera. 4, 5, 6, 7, and 8. Ciliary body; 4, meridional, 5, radial, 6, circular fibers of ciliary muscle; 7, ciliary process; 8, ciliary portion of retina. 9. Iridal portion of retina. 10. Stroma of the iris. 11, 12, and 13. Cornea; 11, posterior elastic lamina; 12, substantia propria; 13, epithelium. 14. Venous sinus of sclera. 15. Angle of iris. Techn. No. 169 a.

inner surface are patches of retinal pigment. The glassy membrane approaches in character the elastic membranes.

The *ciliary body* is formed by the ciliary processes and the ciliary muscle.

The *ciliary processes* are seventy or eighty meridionally-placed folds, which begin low at the ora serrata, gradually attain a height of 1 mm., and terminate with an abrupt descent near the edge of the lens. Each ciliary

FIG. 228.—VERTICAL SECTION OF PUPILLARY PORTION OF HUMAN IRIS. $\times 100$. About one-fifth of the entire width of the iris is shown. *g*. Blood-vessel, with thick connective-tissue sheath; *m*, sphincter pupillae muscle, cut transversely; *p*, pupillary border of the iris. Techn. No. 170 c.

process consists of fibrillar connective tissue containing numerous blood-vessels and inwards is limited by a continuation of the glassy membrane, which here is distinguished by minute intersecting folds.

The *ciliary muscle* is an annular band, about 3 mm. broad, anteriorly 0.8 mm. thick, arising from the inner wall of Schlemm's canal. The nonstriped elements of which it is composed extend in three different directions: (1) *meridional fibers*, numerous fasciculi running parallel to the sclera, which reach to the smooth portion of the choroid and are known as the tensor choroideæ; (2) *radial fibers*, lying next to the meridional bundles, which from without inward progressively assume a more radial disposition (oriented to the center of the bulbus oculi) and posteriorly, still in the region of the ciliary body, turn and follow a circular course; (3) *circular (equatorial) fibers*, the so-called *ring-muscle of Müller*.

The *iris* consists of a stroma arranged in three layers, covered anteriorly by a continuation of the posterior endothelium of the cornea and posteriorly by a modified extension of the retina. Accordingly five layers may be distinguished:—

1. The anterior endothelium.
2. The anterior boundary layer.
3. The vascular layer.
4. The posterior boundary layer.
5. The pigment layer.

The *anterior endothelium* covers the anterior surface of the iris and, like that on the posterior surface of the cornea, consists of a single layer of flattened polygonal cells.

The *anterior boundary layer* (reticular layer) comprises three or four strata of networks, which are formed by stellate connective-tissue cells; it resembles the reticulum of adenoid tissue. The posterior stratum passes gradually into the adjoining vascular stroma.

The *vascular layer of the iris* contains numerous radially-disposed (to the pupil) blood-vessels embedded in a stroma consisting of slender, loosely-united bundles of connective tissue. The blood-vessels and nerves are provided with conspicuously thick connective-tissue sheaths. The smooth muscle-fibers in the vascular stroma are arranged in two sets, as *annular bundles* encircling the pupillary margin of the iris, a zone about 1 mm. in width constituting the *sphincter of the pupil*, and as a few radially-disposed bundles, which do not form a continuous layer—the *dilator of the pupil*. In the anterior boundary layer and in the vascular stroma pigmented cells occur in greatly varying numbers; in blue eyes they are absent.

The *posterior boundary layer* is a clear, glassy, homogeneous membrane, elastic in its nature.

The *pigment layer of the iris* (pars iridica retinæ) comprises two layers, of which the anterior contains spindle-shaped, the posterior polygonal pigment-cells. Both layers are so crowded with pigment-granules that recognition of the individual elements is usually impossible. The pigment is wanting only in albinos. The posterior surface of the pigment layer is covered by an exceedingly delicate membrane, the *membrana limitans iridis*, a continuation of the *membrana limitans interna retinæ*.

The Irido-Corneal Angle.—The juncture of the sclera and the cornea is of especial interest, since here the iris, the cornea, and the ciliary body meet. The transformation of the sclera into the cornea is absolutely direct; the wavy bundles of the sclera, without interruption in continuity, pass over into the straight bundles of the cornea, the system of canaliculi of the sclera communicates with that of the cornea. The line of transition is oblique, and microscopically not sharply defined, because the transformation of the sclera into the tissues of the cornea takes place soonest in the posterior strata of the tunica externa. At the periphery of the cornea the posterior basal membrane and the hindermost laminae of the substance proper meet the ciliary border of the iris and form the *irido-corneal angle*. Here the iris sends toward the posterior surface of the posterior basal membrane connective-tissue processes, that, well developed in animals, constitute the so-called *ligamentum iridis pectinatum*. In man these processes are inconspicuous. At the periphery of the cornea the posterior basal membrane splits up into fibers which, strengthened by elastic fibers from the intramuscular connective tissue of the ciliary muscle and from the elastic ten-

FIG. 229.—VERTICAL SECTION OF HUMAN RETINA. $\times 240$. The nerve-fiber layer has been cut transversely and is very thin, because the section was not taken from the posterior segment of the eye. *b*, Blood-vessel; *k*, expanded base of radial fiber. Techn. No. 170 *d*.

dons, also with accessions in a lesser degree from the sclera, blend with the iridal processes. The tissues participating in the formation of the loose mass of fibers occupying the angle of the iris are derived from the structures that meet one another at the irido-corneal angle: cornea, sclera, iris, and ciliary muscle. The posterior endothelium of the cornea continued on to the surface of the iris forms a sheath for these fibers. The spaces between them, in direct connection with the anterior chamber of the eye and containing the same fluid, are called the *spaces of Fontana*. In man they are but slightly developed.

THE TUNICA INTERNA.

The *retina* extends from the entrance of the optic nerve to the pupillary margin of the iris, and in this tract three zones may be distinguished: (1) the *pars optica retinae*, the entire expansion of the optic nerve; (2) the *pars ciliaris retinae*, extending from the ora serrata to the ciliary margin of the iris; (3) the

pars iridica retinae, which covers the posterior surface of the iris from the ciliary to the pupillary margin.

The *pars optica retinae*, the portion of the retina alone sensitive to light, lines the entire posterior segment of the eyeball and extends to within a short distance of the ciliary body, where it terminates in a sharp, macroscopically perceptible, serrated line, the *ora serrata*. It falls into two divisions, an outer *neuro-epithelial lamina*, and inner *cerebral lamina*. In each of these divisions several layers may be distinguished—four in the neuro-epithelial lamina, five in the cerebral lamina; if the pigment layer lying close beneath the choroid, which genetically belongs to the retina, is added, there are ten layers, which from without inward are arranged in the following order:—

1. The pigment layer.	
2. The layer of rods and cones.	
3. The membrana limitans externa.	} Neuro-epithelial lamina.
4. The outer granule layer.	
5. The fiber-layer of Henle.	} Cerebral lamina.
6. The outer reticular layer.	
7. The inner granule layer.	
8. The inner reticular layer.	
9. The ganglion-cell layer.	
10. The nerve-fiber layer.*	

The elements of the preceding layers are only in part nervous, or epithelial, in their nature; the other part is formed of the supporting substance, which however is not of the nature of connective tissue. The most conspicuous elements of the supporting tissue are the *radial fibers of Müller*, elongated cells which extend from the inner surface of the retina through all the layers to the rods and cones. The inner end of the fibers is characterized by a conical foot, and they are so closely placed that the expanded bases apparently produce a continuous membrane on the inner surface of the retina, the so-called *membrana limitans interna*. From the apex of the pyramidal base the fibers proceed, with progressive decrease in thickness, through the inner reticular layer to the inner granule layer, where they are provided with a nucleus; from here they pass through the outer reticular and outer granule layer to the external limiting membrane, with which they unite. Throughout their entire course the radial fibers give off lateral processes for the support of the nervous elements. In addition to these elongated radial cells, *concentric supporting cells* are found in the outer reticular layer: they extend parallel to the surface, are provided with long processes, are in part nucleated, in part nonnucleated. From the surface of the *membrana limitans externa* delicate processes extend to the rods and cones, the bases of which they embrace as the so-called *fibrocrates*. A portion of both the reticular layers belongs to the supporting sub-

* To these the *membrana limitans interna* is sometimes added as an eleventh layer; it, however, does not represent an independent membrane.

stance, and also the small quantity of cement-substance in the ganglion-cell layer.

In the more detailed description of the individual layers of the retina the series will be taken up in the reverse order, from within outward.

THE CEREBRAL LAYER.

The *nerve-fiber layer* consists of naked axis-cylinders arranged in bundles and united in a sort of plexus. From the entrance of the optic nerve, where the fiber-layer is thickest, the fibers extend outward in a radial direction to the ora serrata. The radial arrangement of the fibers is disturbed in the region of the macula lutea. The majority of the axis-cylinders are centripetal fibers which originate in the ganglion-cell layer of the retina; the smaller portion are the axis-cylinder processes of cerebral ganglion-cells, centrifugal fibers, which ramify in the inner nuclear layer and terminate in free endings.

The *ganglion-cell layer* consists of a single row of large multipolar ganglion-cells, whose unbranched axis-cylinder processes are centrally directed, toward the nerve-fiber layer, whose one or more branched protoplasmic processes ex-

FIG. 230.—VERTICAL SECTION OF RETINA OF RABBIT. $\times 240$. *k*, Expanded base of radial fibers; *n*, nucleated portion of the same; *l*, "membrana limitans interna." Techn. No. 170 *d*.

tend peripherally, toward the inner reticular layer; there they divide and are arranged in delicate feltworks parallel to the surface, and with the processes from other ganglion-cells form a dense nervous tangle.

The *inner reticular layer* consists of an exceedingly delicate network of sustentacular tissue, which supports a dense fiber-maze in the formation of which processes of all the ganglion-cells of the retina participate.

The *inner granule layer* includes elements which differ greatly in their nature. The innermost stratum consists of large ganglion-cells,* which send branched processes into the inner reticular layer. From many of these cells—but not all—an axis-cylinder process passes to the nerve-fiber layer. The re-

* These cells were formerly called spongioblasts, because they were erroneously regarded as the producers of the "neuro-spongium" (inner reticular layer); they are elements of the ganglion of the optic nerve which have not, like the other elements, wandered through the inner reticular layer.

maining strata, for the greater part, are composed of small bipolar ganglion-cells (ganglion retinæ), whose central process extends into the inner reticular layer, where it breaks up into delicate varicose branches; while the peripheral process reaches to the outer reticular layer, where it divides into branches extending parallel to the surface and resolves into extremely minute fibrillæ, which pass into a subepithelial tangle formed by the felting of processes of neighboring ganglion-cells. All bipolar ganglion-cells send up a process between the visual cells, where, near the membrana limitans, it terminates in a minute knob. The nuclei of the radial fibers occur in this layer.

FIG. 231.—SCHEME OF THE ELEMENTS OF THE RETINA; the figure on the left represents the supporting elements, that on the right the nervous and epithelial elements.

At the border of this zone, next to the outer reticular layer, lie small and large stellate cells; they send many processes to participate in the formation of the subepithelial network; one process extends to the inner reticular layer, where it terminates in minute branches, and another—the axis-cylinder process—after a long horizontal course, bends and passes in a vertical direction to the nerve-fiber layer. According to other authors this process ends in the outer reticular layer, where its ramifications surround the base of the visual cells.

The *outer reticular layer* (subepithelial layer) is likewise a delicate network of sustentacular tissue, which supports the nervous tangle just described. The cellular elements of this layer include the concentric sustentacular cells and the subepithelial ganglion-cells; the latter are displaced elements of the ganglion retinæ, which differ from the bipolar ganglion-cells only in their

rounded form, agreeing entirely with the latter in regard to their terminal ramifications.

THE NEURO-EPITHELIAL LAYER.

The neuro-epithelial layer consists of two kinds of elements, the *rod-visual cells* and the *cone-visual cells*, which are both characterized by the situation of the nucleus in the lower half of the cell and the sharp demarcation of the upper nonnucleated division from the lower portion by the perforated *membrana limitans externa*. This gives rise to the appearance of different layers, the inner nucleated portion of the visual-cells being known as the outer granule layer, the outer nonnucleated division as the layer of rods and cones. Between these two lies the limiting membrane.

The Rod-Visual Cells.—The outer halves of these elements are the *rods*, slender cylinders ($60\ \mu$ long, $2\ \mu$ thick), which consist of a homogeneous outer segment and a finely-granular inner segment. The outer segment is

FIG. 232.—ISOLATED ELEMENTS OF RETINA OF APE. $\times 240$. 1. Mutilated ganglion-cell of the ganglion of the optic nerve. 2. Elements of the inner granule layer. 3. Rod-visual cells and fragments of the same; below, two outer segments, one of which exhibits transverse striation, the beginning of a disintegration into transverse platelets; above are two rods, the outer segment of the lower shows beginning disintegration; the uppermost figure shows more complete rod-cells; *a*, outer segment; *i*, inner segment; *k*, nucleus of rod; *x*, fiber-body. 4. Cone-visual cells; *a*, outer segment; *i*, inner segment; *k*, nucleus of cone; *f*, cone-fiber, torn at lower end; *x*, fiber-body. 5. Radial fiber; *k*, nucleus of the same; *r*, expanded base of radial-fiber. Techn. No. 172.

the exclusive seat of the *visual purple*. The inner segment possesses in its outer end an ellipsoidal, fibrillated body, the *fiber-body*. The inner halves of the rod-visual cells are named *rod-fibers*; they are exceedingly delicate filaments which are provided with nucleated expansions, the *rod-granules*. The nuclei are marked by one to three light transverse bands. The basal end of the cell is prolonged as a minute process, terminating in a free, club-shaped expansion (Fig. 231).

The Cone-Visual Cells.—The outer halves of these cells, the *cones*, consist likewise of an outer segment and inner segment. The outer segments are conical and shorter than those of the rods. The inner segments are thick and expanded, and the cone is therefore flask-shaped. The inner segment of the cones also contains a fiber-body. The inner halves of the cone-visual cells are the *cone-fibers*; these are broad and rest with a pyramidal expansion or foot on the outer reticular layer. The nucleated enlargement, the *cone-granule*, usually lies to the inner side of, and close to, the *membrana limitans*.

FIG. 233.—HORIZONTAL SECTION THROUGH THE MACULA AND THE CENTER OF FOVEA OF A MAN SIXTY YEARS OLD. (From a preparation by Prof. Haab, drawn by Schaper.) $\times 135$. The nerve-fiber layer, like all the layers, is thicker on the side toward the entrance of the optic nerve than on the opposite side; in the latter situation the nerve-fibers in transverse section appear as minute dots.

The number of the rods is much greater than that of the cones. The latter occur at regular intervals, so that three to four rods lie between two cones (Fig. 229).

The basal portions of the visual cells, resting upon the outer reticular layer, are usually plainly to be recognized as a peculiar radially-striated layer—*Henle's fiber-layer*; in the region of the macula lutea this fiber-layer is particularly broad; it gradually diminishes—often very unsymmetrically—toward the ora serrata.

The *pigmented epithelium* consists of a simple layer of hexagonal cells, which in the outer zone, toward the choroid, where the nucleus lies, are free from pigment, while their inner division contains numerous rod-shaped pigment-granules, 1 to 5 μ long. From the inner division numerous delicate processes extend between the rods and cones. In albinos and on the tapetum the epithelium is free from pigment.

In the region of the macula lutea and fovea centralis, also of the ora serrata, the structure of the retina above described presents modifications calling for special consideration.

At the macula the layers of the retina exhibit the following variations. Delicate fibers of the optic nerve run direct from the entrance of the latter to the adjacent median portion of the macula; above and below these fibers, thicker nerve-fibers run from the optic entrance convexly upwards and downwards and unite at the lateral margin of the macula. The ganglion-cell layer has greatly increased in thickness, owing to the development of the layer of bipolar ganglion-cells, which instead of a single row are arranged in many (up to 9) rows one above the other; also the inner granule layer by multiplication of its elements has become almost twice as broad. The inner and outer granule layers suffer no essential change. The neuro-epithelial layer is here represented by the somewhat smaller cone-visual cells alone. The rod-visual cells diminish in number at the margin of the macula, and within the macula they are wanting altogether; as a result the cone-fibers are visible in a wide extent: they form here the fiber-layer of Henle. The cone-granules, on account of their large number, lie in several rows one above the other.

Toward the *fovea centralis* in the center of the macula the layers of the retina become gradually thinner and in part totally suspended. At first, with the exception of a few fibers, the nerve-fiber layer disappears, then the cerebral layers fuse with one another and in the center of the fovea with the cone-granules, forming a thin layer in which the boundaries of the individual strata can no longer be recognized. In the center of the fovea (*fundus foveæ*) the neuro-epithelial layer (cone-cells) alone is present.

A diffuse yellow pigment permeates the cerebral layer, but is absent in the neuro-epithelial layer, and therefore the *fundus foveæ* is colorless.

In the region of the *ora serrata* a rapid diminution in the retinal layers takes place. Optic-fibers and ganglion-cells disappear before reaching the ora serrata. Of the visual cells the rod-visual cells are the first to vanish; the cone-visual cells are still preserved, but appear to be deprived of their outer

segments. Then the outer reticular layer is lost, so that the outer and inner granular layers become confluent, and finally, the inner reticular layer ceases. The radial fibers of Müller, on the contrary, persist and are highly developed. The ora serrata is frequently the seat of senile change. Commonly vacuoles occur; they appear first in the outer granule layer, and may extend into the central layers.

The *pars ciliaris retinæ* consists of a simple layer of slender columnar cells, which gradually originate in the blended inner and outer granule layers. These cells are covered on their centrally-directed surface by a cuticular membrane, a true *membrana limitans interna*, which is not present in the *pars optica retinæ*; their peripheral surface is joined to pigmented cells, a continuation of the pigmented epithelium.

The *pars iridica retinæ*, the pigment layer of the iris, has been described (p. 241).

With regard to the *connections of the nervous elements of the retinæ*, according to the foregoing description the axis-cylinder processes of the ganglion-cells of the ganglion of the optic nerve (basal optic ganglion) and of

FIG. 234.—MERIDIONAL SECTION OF THE ORA SERRATA AND THE ADJACENT PORTION OF THE PARS CILIARIS RETINÆ OF A WOMAN SEVENTY-EIGHT YEARS OF AGE. $\times 70$. 1. Pigmented epithelium. 2. Cones, minus their outer segment. 3. External limiting membrane. 4. Outer granule layer. 5. Outer reticular layer. 6. Inner granule layer. 7. Inner reticular layer. 8. Radial fibers. 9. Vacuole in the retina. At 10 the outer and inner nuclear layers become confluent and at 11 continuous with the cells of the *pars ciliaris retinæ*. Techn. No. 179 d.

the stellate cells of the inner granule layer are the centripetal optic-fibers; while the centrifugal nerve-fibers terminate in free endings in the inner granule layer. The ganglion-cells of the ganglion retinae apparently possess no axis-cylinder processes; their union with the other nervous elements is effected by means of the nervous tangles in the two reticular layers (Fig. 231). It has recently been positively asserted that a *direct* connection exists between the nervous elements of the retina, established by broad anastomoses and by a true network. The connection with the visual cells is effected by the intraepithelial processes of the cells of the ganglion retinae, which terminate between (not within) the visual elements. Physiologic researches make it highly probable that the visual-cells constitute the essential percipient part of the retina.

THE OPTIC NERVE.

The *optic nerve*, in its entire intraorbital course, is enveloped in sheaths which are processes of the cerebral membranes. Outermost is the compact dural sheath, consisting of longitudinally-disposed bundles of connective-tissue;

following this is the exceedingly delicate arachnoidal sheath, which sends numerous relatively thick connective-tissue trabeculae inward to the pial sheath, while the union with the dural sheath is represented by a few delicate fibers. Innermost lies the pial sheath, which closely invests the optic nerve and sends off numerous septa between the individual nerve-fiber bundles. These septa are connected with one another by transverse trabeculae, the resultant structure being a transverse lattice-work.

The tissue of the pial sheath does not penetrate within the nerve-fiber bundles, but only forms an outer envelope for them. The nerve-fiber bundles consist of medullated fibers without a neurilemma; they are held together by many neuroglia-cells ("mossy" cells). At the entrance of the optic-nerve into the eyeball the dural sheath passes into the sclera, the arachnoidal sheath, at

FIG. 235.—LONGITUDINAL SECTION OF OPTIC ENTRANCE OF HUMAN EYE. $\times 15$. Above the lamina cribrosa the narrowing of the optic nerve is visible. The central artery and vein have been for the most part cut longitudinally, but above at several points, transversely. Techn. No. 169 d.

its anterior border, breaks up into fibers, so that the subdural space lying to its outer side communicates with the subarachnoidal space on its inner side. The pial sheath blends with the sclera, which here is pierced with numerous apertures for the nerve-fibers passing through it; this portion of the sheath is called *lamina cribrosa*. The choroid also participates, though in a slight degree, in the formation of the lamina cribrosa. The nerve-fibers lose their medullary sheaths at the point of entrance, and consequently the nerve becomes considerably reduced in size.

In the distal half of the optic nerve, the central artery and vein of the retina lie in its axis; the connective-tissue investing these vessels is connected at many points with the pial sheath, as well as with the lamina cribrosa.

THE LENS.

The crystalline lens consists of a *substantia propria* which on its anterior surface is covered by the epithelium of the lens; the whole is enveloped by the lens-capsule.

In the *substantia propria* a soft cortical substance and a firm *core* may be distinguished; it consists throughout of colossal, greatly-elongated epithelial-cells, the *lens-fibers*. They have the form of six-sided prismatic bands, which are thickened at their posterior extremities. The lens-fibers of the cortical zone have smooth borders, and in the vicinity of the equator lies an oval nucleus. The lens-fibers of the central portion of the lens have dentated outlines and are without nuclei. All the fibers are united and held together by a small amount of cement-substance, which is accumulated in larger quantities at the anterior and posterior poles of the lens and produces the so-called anterior and posterior *lens-stars*, stellate forms seen in macerated preparations. All the lens-fibers, beginning at the anterior lens-star, run in a meridional direction to the posterior lens-star; but no lens-fiber spans the entire half of the lens; the nearer the fibers arise to the anterior pole, the more remote from the posterior pole do they find their termination.

FIG. 236.—LENS-FIBERS OF AN INFANT. *A*. Isolated lens-fibers, three with smooth, one with dentated borders. $\times 240$. Techn. No. 178. *B*. Human lens-fibers cut transversely; *c*, section through club-shaped ends. $\times 560$. Techn. No. 179.

FIG. 237.—CAPSULE AND EPITHELIUM OF ADULT HUMAN LENS. *C*. Inner aspect, $\times 240$. Techn. No. 180 *a*. *D*. Lateral aspect, from a meridional section through the equator of the lens; 1, capsule, 2, epithelium, 3, lens-fibers. $\times 240$. Techn. No. 180 *b*.

The *lens-epithelium* consists of a simple layer of cubical cells, which covers the anterior surface of the lens and extends as far as the equator; here the

epithelium, by gradual elongation of its elements, becomes transformed into the lens-fibers (Fig. 237, *D*).

The *lens-capsule* is a transparent, glassy, elastic membrane; the *anterior capsule*, the portion covering the anterior surface of the lens, is 11 to 15 μ thick, the corresponding posterior portion, the *posterior capsule*, only 5 to 7 μ . The lens-capsule comprises two genetically distinct parts; the one is a cuticular formation, a product of the epithelium of the lens, the other, of the nature of connective tissue, is a transformation product of the embryonal connective-tissue sheaths.

THE VITREOUS BODY.

The *vitreous body* consists of a fluid substance—the *vitreous substance*—and of *fibers* which extend in all directions through the former. The surface of the vitreous body is covered by a somewhat firmer membrane, the *hyaloid membrane*, and in certain localities contains a limited number of fibrillæ and a few cells; of the latter two forms may be distinguished, round elements, resembling leucocytes, and stellate or fusiform cells. Cells containing clear vacuoles are probably degenerating forms.

THE SUSPENSORY LIGAMENT.

The *suspensory ligament* (zonula ciliaris, zone of Zinn), consists of delicate homogeneous fibers which extend from the surface of the hyaloid membrane, in the vicinity of the ora serrata, in a meridional direction toward the lens. They are attached to the inner surface of the ciliary processes and extend from the apices of the same over to the equator of the lens, where they are attached to the anterior and posterior surfaces and to the equator of the lens-capsule. The fibers do not form a continuous membrane, but are radially plicated extensions of the hyaloid membrane that find attachment and support on the lens. The annular cleft between the zonula ciliaris behind and the vitreous body in front is designated *canal of Petit*. Other authors describe the triangular space included between the anterior and posterior zonula fibers and the lens-capsule as the canal of Petit. The canal is not completely closed on the side toward the posterior chamber of the eye.

THE BLOOD-VESSELS OF THE EYEBALL.

The blood-vessels of the eyeball are separated in two sharply-defined regions, which are in communication only at the entrance of the optic nerve.

Territory of the Vasa Centralia Retinæ.—The *central artery of the retina*, at a distance of 15 to 20 mm. from the eyeball, enters the axis of the optic nerve and runs within it to the surface of the optic entrance. Here it divides into two main branches, of which the one is directed upward, the other downward, each of which subdivides and supplies the entire pars optica retinæ to the ora serrata. During its course in the optic nerve the artery gives off numerous small branches, which run within the processes of the pial sheath

between the nerve-fiber bundles, and anastomose with small arteries that have entered the sheath of the nerve from the surrounding adipose tissue and also with twigs from the short ciliary arteries. In the retina itself the artery breaks up into capillaries, which extend into the outer reticular layer. The cerebral layer of the retina alone contains blood-vessels; in the fundus foveæ the cerebral layer is wanting, and with it the blood-vessels. The veins proceeding from the capillaries run parallel with the branches of the arteries and finally unite in the *vena centralis retinæ* enclosed within the axis of the optic nerve (Fig. 238).

In the embryo a twig from the central artery of the retina, the *hyaloid artery*, passes through the vitreous body to the posterior surface of the lens. This artery atrophies before birth, but the canal which transmits it may still be found in the vitreous body of the adult; it is called the *hyaloid canal*.

Territory of the Vasa Ciliaria.—This region is characterized by the complementary veins taking a course entirely different from that of the arteries.

Of the *arteries*, the short ciliary arteries supply the smooth portion of the choroid, while the long ciliary arteries and the anterior ciliary arteries are destined chiefly for the ciliary body and the iris.

The branches, about twenty, of the *short ciliary arteries* penetrate the sclera in the vicinity of the optic entrance; after giving off twigs which supply the posterior half of the surface of the sclera, the arteries break up into a narrow-meshed capillary network, the *choriocapillaris*. At the optic entrance the arteries anastomose with branches of the *arteria centralis retinæ* and there form the *circular artery of the optic nerve*; at the *ora serrata* they anastomose with recurrent twigs of the long ciliary and the anterior ciliary arteries.

The two *long ciliary arteries* likewise penetrate the sclera at the optic entrance; the one artery passes to the nasal, the other to the temporal side of the eyeball between the choroid and the sclera to the ciliary body, where each artery divides in two diverging branches running along the ciliary margin of the iris; by the anastomoses of the branches of the two arteries a vascular ring is formed, the *larger arterial circle* of the iris (*circulus iridis major*) from which numerous twigs are given off to the ciliary processes and the iris. Near the pupillary margin of the iris the arteries form an incomplete ring, the *smaller arterial circle* (*circulus iridis minor*).

The *anterior ciliary arteries* come from the arteries supplying the recti muscles of the eye, penetrate the sclera near the corneal margin, communicate with the larger arterial circle of the iris, supply the ciliary muscle, and send recurrent branches to unite with the choriocapillaris. Before the anterior ciliary arteries penetrate the sclera, they give off twigs *behind* for the anterior half of the sclera, and *in front* to the conjunctival sclera and to the corneal limbus. The cornea itself is without blood-vessels; only at the margin, in the anterior lamellæ of the substantia propria, is there a circumferential network of capillary loops.

The *veins* all run toward the equator, where they converge to four (more rarely five or six) small stems, the whorl veins or *venæ vorticosæ*, which forth-

with pierce the sclera and empty into one of the ophthalmic veins. In addition to these there are small complemental veins that run parallel to the short ciliary arteries and the anterior ciliary arteries; the anterior ciliary veins receive twigs

FIG. 238.—SCHEME OF THE VESSELS OF THE EYE, ACCORDING TO LEBER. External tunic stippled, middle tunic white, internal tunic and optic nerve dotted crosswise. Arteries light. Veins dark. Region of the central vessels of the retina (small Italic letters): *a*, Artery; *a'*, central vein of retina; *b*, anastomosis with vessels of the sheath; *c*, anastomosis with branches of the posterior short ciliary arteries; *d*, anastomosis with choroidal vessels. Region of the vessels of the sheath (large Italic letters): *A*, Inner; *B*, outer vessels of the sheath. Region of the posterior short ciliary vessels (Italic numerals): *I*, Arteries; *I'*, veins (short posterior ciliary); *II*, episcleral arterial; *II'*, episcleral venous branches of the same; *III*, capillaries of the choriocapillaris. Region of the posterior long ciliary vessels (Arabic numerals): *1*, Posterior long ciliary artery; *2*, circulus iridis major cut transversely; *3*, branches to the ciliary body; *4*, branches to the iris. Region of the anterior ciliary vessels (Greek letters): *α*, Artery; *α'*, vein (anterior ciliary); *β*, connection with the circulus iridis major; *γ*, connection with the choriocapillaris; *δ*, arterial; *δ'*, venous episcleral branches; *ε*, arterial; *ε'*, venous branches to the scleral conjunctiva; *η*, arterial; *η'*, venous branches to the corneal limbus; *I'*, vena vorticosæ; *S*, cross-section of the venous sinus of the sclera.

from the ciliary muscle, from the episcleral vascular network, from the conjunctival sclera, and from the circumferential capillary loops of the cornea. The episcleral veins communicate with the venæ vorticosæ at the equator.

The anterior ciliary veins eventually communicate also with the canal of Schlemm. This canal is an annular cleft encircling the cornea, but lying just within the sclera. It is by some regarded as a lymph-space in open communication with the anterior chamber, by others held to be a venous channel.

THE LYMPH-CHANNELS OF THE EYEBALL.

The eye possesses no proper lymph-vessels, but a series of intercommunicating lymph-spaces. Two complexes of such spaces may be distinguished, an anterior and a posterior tract. The anterior tract comprises:—

1. The *lymph-canalliculi* of the *cornea* and *sclera*.
2. The *anterior chamber* of the eye, which, with Schlemm's canal, by means of the capillary clefts between the iris and the lens, communicates with—
3. The *posterior chamber* of the eye. The latter is in open connection with—
4. The *canal of Petit*.

The last three spaces stand in close relation to one another, and may be injected from the anterior chamber.

The posterior tract includes:—

1. The *hyaloid canal*.
2. The *lymph-clefts* between the sheaths of the *optic nerve* (the subdural and the subarachnoid spaces), the narrow cleft between the choroid and the sclera—the perichoroidal space—and Tenon's space, which extends from the dural sheath of the optic nerve to the optic foramen. These spaces may be filled from the subarachnoid space of the brain. The contents of these spaces is a filtrate from the blood-vessels, which also permeates the vitreous body. The quantity of this fluid in the perichoroidal space, also in Tenon's space, is normally exceedingly scanty. Both these spaces serve to facilitate the movements of the choroid and of the eyeball, and may be regarded as synovial spaces.

THE NERVES OF THE EYEBALL.

The nerves of the eyeball penetrate the sclera in the vicinity of the entrance of the optic nerve and run forward between the outer tunic and the choroid; after giving off bundles accompanied by ganglion-cells to the choroid, they form an annular plexus intermingled with ganglion-cells lying upon the ciliary body—the *ciliary ganglionic plexus* (*orbiculus gangliosus ciliaris*), from which branches go to the ciliary body, the iris, and the cornea. The nerves of the *ciliary body* terminate in delicate pointed ends in the blood-vessels, in the ciliary muscle between the muscle-bundles in the form of branched ends, which perhaps subserve the muscular sense, and on the scleral surface of the ciliary body in the form of a delicate plexus. The *medullated nerves of the iris* form networks and lose their medullary sheath as they pass to the pupillary margin: their terminal ramifications are in part distributed to the smooth muscle-fibers and the blood-vessel walls; another portion forms a dense sensory plexus lying close

beneath the anterior iridal surface. The nerves to the *cornea* first enter the sclera and form a circular plexus—*plexus annularis*—surrounding the corneal margin, from which branches are distributed to the sclera and to the cornea. In man the twigs in the sclera terminate in spherical end-bulbs lying close under the epithelium; they are also found in the substance proper of the cornea for a distance of from 1 to 2 mm. within the corneal limbus. The branches that go to the cornea, after their entrance in the substance proper lose their medullary sheath and as naked axis-cylinders penetrate the entire structure. They form networks, which, according to the plane they occupy, are described as—the *stroma* or *ground-plexus*, which lies in the deeper strata of the cornea; the *subbasilar plexus*, situated beneath the anterior basal membrane; the *sub-epithelial plexus*, lying close under the epithelium. From the latter plexus exquisitely-delicate nerve-fibrillæ pass up into the epithelium between its elements, and form the exceedingly fine *intraepithelial plexus*, whose naked axis-cylinders terminate in free ends between the epithelial cells (Fig. 239).

FIG. 239.—FROM A VERTICAL SECTION THROUGH THE HUMAN CORNEA. $\times 240$. *n*, Point of division of nerve penetrating the anterior basal membrane; *s*, subepithelial plexus beneath the cylindrical cells; *a*, fibers of the intraepithelial plexus ascending between the epithelial cells. Techn. No. 177.

THE EYELIDS.

The eyelids are folds of the integument, which enclose muscles, loose and compact connective tissue, and glands. The outer fold of the eyelid retains the usual characteristics of the skin; the inner fold, that toward the eye, is considerably modified and is called the *palpebral conjunctiva*. The skin on the external surface of the eyelid extends over the lower free margin and does not pass into the palpebral conjunctiva until it reaches the posterior border, *palpebral border*.

The eyelid is best studied in a sagittal section, in which, counting from before backward, the following strata are found:—

1. The *integument* is thin and beset with fine hairs; in the corium small sweat-glands are found, also pigmented connective-tissue cells: the latter are of rare occurrence in the corium elsewhere. The subcutaneous tissue is very loose, rich in fine elastic fibers, and contains but few fat-cells, which may be entirely wanting. Near the border of the lid the corium is more compact and beset with more conspicuous papillæ. At the anterior edge of the margin of the lid two to three rows of robust hairs, the *cilia*, extend obliquely outward; their follicles are deeply implanted in the corium. The cilia undergo rapid shed-

ding; their length of life is said to be about from 100 to 150 days; as a result, hairs in all stages of development are frequently found in the eyelashes. The hair-follicles of the cilia are provided with small sebaceous glands, and take up the excretory ducts of the so-called *Moll's glands*, which in their minute structure resemble coil-glands, and differ from these only in that their lower ends are less convoluted.

2. Posterior to the subcutaneous tissue lie transverse bundles of cross-striated muscle-fibers of the orbicularis palpebrarum; the portion of the muscle lying behind the cilia is named the *ciliary* or *marginal muscle*.

FIG. 240.—SAGITTAL SECTION OF THE UPPER EYELID OF A SIX-MONTHS-OLD CHILD. $\times 10$. 1. Integument: *E*, epidermis; *C*, corium; *Sc*, subcutaneous tissue; *Hb*, hair-follicle of lanugo hair; *K*, coil-gland. *W*, eyelash, with the anlage of a new hair (*Eh*). *W'*, *W''*, portions of follicles of eyelashes; *M*, portion of a gland of Moll. 2. Region of the orbicularis palpebrarum muscle: *O*, bundles of this muscle cut transversely. *McR*, lid muscle. 3. Expanded tendon of the levator palpebrarum superior; *mpz*, superior palpebrarum muscle. 4. Conjunctival portion: *e*, conjunctival epithelium. *tp*, tunica propria; *at*, accessory tear-glands. *t*, tarsus; *m*, Meibomian glands, the mouth of the excretory duct is not visible. *a*, transverse section of the arcus tarsus. *a'*, transverse section of the arcus tarsus externus. 5. Margin of eyelid. Techn. No. 182.

3. Behind the muscle the fibrous extensions of the tendon of the levator palpebræ are met, which are partly lost in the areolar tissue present—the fascia palpebralis,—and partly attached to the upper margin of the tarsus: the latter contain smooth muscle-fibers, the *lid-muscle of Müller*. In the lower eyelid the expansion of the tendon of the inferior rectus muscle contains bundles of nonstriated muscle-fibers.

4. The *tarsus* is a plate of dense fibrous tissue, which gives firmness and support to the eyelid. It lies immediately in front of the conjunctiva, to which it belongs, and occupies the entire lower two-thirds of the height of the eyelid. In its substance the Meibomian glands are embedded, elongated bodies which consist of a wide excretory duct, opening on the palpebral border, and of short acini. In their histology the Meibomian glands agree with the sebaceous glands. At the upper edge of the tarsus lie branched tubular glands, in part enclosed by its substance, which in their minute structure coincide with the tear-glands, and therefore are called *accessory tear-glands*; they occur principally in the inner (nasal) half of the eyelid.

Behind the tarsus lies the *conjunctiva* proper, which consists of an epithelium and a tunica propria. The former is a stratified columnar epithelium, with several rows of spherical cells in the deeper portion and a row of mainly short cylindrical cells on the surface. The latter possess a narrow hyaline cuticular border. Goblet-cells also occur in varying numbers. At the palpebral border the epithelium passes gradually into the stratified scaly variety, which occasionally extends far over on the conjunctiva. The lower portion of the palpebral conjunctiva is smooth. In the upper portion, on the contrary, the epithelium forms irregular *pocket-like depressions*, which differ greatly in individual development and in sections, when highly developed, resemble glands. The tunica propria of the conjunctiva consists of fibrous tissue, plasma-cells, in varying number, and of lymphoid cells, whose number likewise varies greatly. In animals, especially in ruminants, the latter form true nodules, the so-called *trachoma* glands, from the summit of which the leucocytes wander through the epithelium to the surface; in man, the migration of the leucocytes occurs in a slighter degree. In the region of the conjunctival recesses, the tunica propria is divided into papillæ by the depressions of the epithelium.

The palpebral conjunctiva passes from the eyelid to the eyeball, the anterior surface of which it covers. At the line of transition, the *fornix conjunctivæ*, a loose sub-conjunctival tissue occurs under the tunica propria. The epithelium is the same as that on the lid; the tunica propria contains fewer leucocytes, but possesses normally about twenty small lymph-nodules and a few mucous glands. On the scleral conjunctiva the stratified columnar epithelium, within a certain distance of the cornea, becomes transformed into the stratified scaly variety, which continues over the cornea.

The rudimentary *third eyelid* (*plica semilunaris*) consists of connective tissue and stratified squamous epithelium. The *caruncula lacrymalis* resemble the skin in structure—with the exception that the stratum corneum is absent—and contain fine hairs, sebaceous glands, and accessory tear-glands.

The *blood-vessels* of the eyelids pass from the outer and inner angles, and form an arch, the *arcus tarseus*, at the margin of the lid, and a second arch, the *arcus tarseus externus*, at the upper edge of the tarsus. Branches from these arches ramify in the skin, surround the glands of Meibom, penetrate the tarsus, and form a capillary network lying beneath the conjunctival epithe-

lium; they also supply the fornix conjunctivæ, the scleral conjunctiva, and anastomose with the anterior ciliary arteries.

The *lymph-vessels* form a close-meshed network in the tarsal conjunctiva, a very open-meshed network on the anterior surface of the tarsus. According to some authors, the lymph-channels of the scleral conjunctiva are closed at the corneal limbus; according to others, they send minute canaliculi into the tissue of the cornea, and are thus in communication with the system of lymph-spaces and canaliculi in the latter.

The *nerves* form a rich plexus at the margin of the lid.

FIG. 241.—FROM A THIN SECTION OF HUMAN LACRYMAL GLAND. $\times 240$. *A*, Gland, *a*, tubule cut transversely; *a'*, group of tubules, mostly cut obliquely, the lumen of one tubule only visible below; *s*, intercalated tubule with cubical (above to the left), flat (below to the right), epithelial cells; *a'*, intercalated tubule in cross-section, lined with moderately high cylindrical cells; *b*, connective tissue. *B*, Cross-section of the duct; *c*, double layer of cylindrical epithelium; *b*, connective tissue. Techn. No. 183.

THE LACRYMAL GLANDS.

The *lacrymal glands* are compound tubular glands, provided with several excretory ducts. The latter are clothed with a two-layered cylindrical epithelium, and pass into long narrow intercalated tubules clothed with low epithelial cells. These pass into the gland-tubules, which are lined by serous gland-cells.

The walls of the *lacrymal canaliculi* consist of stratified scaly epithelium and for the greater part of longitudinally-disposed cross-striped muscle-fibers. The tunica propria is rich in elastic fibers and, beneath the epithelium, in cellular elements.

The lacrymal sac and the naso-lacrymal duct are composed of a two-layered columnar epithelium; the tunica propria is chiefly adenoid in character, and is separated from the underlying periosteum by a dense plexus of veins.

XI. THE ORGAN OF HEARING.

The organ of hearing consists of three divisions: the innermost, the *internal ear*, which encloses the end-apparatus of the auditory nerve; the other divisions, *middle ear* and *external ear*, are only accessory apparatus.

THE INTERNAL EAR.

The internal ear consists of two membranous sacs that communicate with each other by means of a minute canal, the *ductus endolymphaticus*. The one saccule, the *utricle*, is in connection with membranous tubules, the *semicircular canals*, each of which at the point where it opens in the utricle possesses a dilatation, the *ampulla*. The other saccule, the *sacculus*, connects with a long spirally-wound membranous duct, the *cochlea*.

The sacculus and utriculus, the semicircular canals and the membranous cochlea constitute the *membranous labyrinth*. This is enclosed within the sphenoid, in a space having similar outlines, the *bony labyrinth*, which it does not completely fill. The unfilled space is occupied by a watery fluid, the *perilymph*. A similar fluid, the *endolymph*, is found within the interior of the membranous labyrinth.

FIG. 242.—OTOLITHS
FROM THE SACCULUS
OF AN INFANT. $\times 560$.
Techn. No. 184

The saccules and the semicircular canals exhibit the same structure, but the cochlea is essentially different, so that it requires a separate description.

The Saccule, the Utricle, and the Semicircular Canals.—Their walls comprise three layers. The outermost is a connective-tissue layer rich in elastic fibers; this is followed within by a delicate basement membrane beset with minute excrescences, which is covered on its inner surface by a simple squamous epithelium. This simple structure undergoes alteration at the positions where the filaments of the auditory nerve are received, the *macula cribrosa* of the saccule and utricle, the *crista acustica* on the ampullæ of the semicircular canals. The connective tissue and basement membrane become thicker here; the squamous epithelium in the vicinity of the maculae and cristæ becomes transformed into columnar epithelium with a cuticular border, and passes into the neuro-epithelium of the maculae and cristæ. The neuro-epithelium is likewise a simple layer, and consists of two kinds of cells: (1) *fiber-cells*, elongated elements that occupy the entire depth of the epithelium, are slightly expanded at the upper as well as at the lower end, and contain an oval nucleus; they are the sustentacular elements; (2) *hair-cells*, cylin-

dric elements occupying only the upper half of the thickness of the epithelium, which in the lower rounded portion contain a spherical nucleus and bear on their free surface a bundle of long, delicate agglutinated filaments, the "auditory hairs." The hair-cells are the terminal apparatus of the auditory nerve. The nerve-fibers lose their medullary sheaths on entering the epithelium, divide, and ascend to the base of the hair-cells as naked axis-cylinders where each fiber divides into three to four varicose twigs, which run beneath several hair-cells parallel to the surface of the epithelium, and finally turn upward and terminate in contact with the lateral surface of a hair-cell in a free pointed end.* During their horizontal course they send upward a few twigs, which end in the same manner in contact with the hair-cells. These ends do not reach to the surface of the epithelium. The free surface of the neuro-epithelium is covered by a continuation of the cuticular zone, which is perforated by the auditory hairs. The maculae acusticae are covered by a soft, gelatinous substance (a cuticula?), in which innumerable prismatic crystals of calcium carbonate, the *otoliths*, 1 to 15 μ in size, are embedded; they form the *otolith membrane*. On the cristae acusticae the so-called *cupola* occurs; in fresh preparations it is an invisible substance; on the application of fixation fluids it coagulates and thus becomes visible.

The wall of the bony labyrinth is covered by a thin periosteum and flattened connective-tissue cells. The saccules and semicircular canals are secured to the walls of the bony labyrinth by means of connective-tissue trabeculae.

THE COCHLEA.

The membranous cochlea, the *ductus cochlearis*, does not entirely fill the space within the bony cochlea. It lies with one wall in contact with the outer wall† of the bony cochlea; the upper or vestibular wall (*membrane of Reissner*) bounds the scala vestibuli; the lower or tympanic wall (*membranous spiral lamina*) is directed toward the scala tympani. The angle in which the vestibular and tympanic wall meet lies on the free end of the osseous spiral lamina. There the periosteum and the fibrous coat of the ductus cochlearis are especially well developed and form a prominence, the *limbus*, which rests with a broad surface on the bony spiral lamina, slopes upwards, and terminates in a sharp edge. This edge is called the *labium vestibulare*; the free margin of the bony spiral lamina is called the *labium tympanicum*; between the labia is a recess, the *sulcus spiralis* (Fig. 249). The inner surfaces of the ductus cochlearis are covered by an epithelium that varies greatly in different localities; the outer surfaces—toward the scala vestibuli and scala tympani—are covered by a deli-

* The horizontal branches interlace and form a small, but direct "lattice-work," which also in other methods than that of Golgi appears to consist of a layer of strongly-refracting granules. The granules are the varicosities and the optical cross-sections of the horizontal fibers.

† I follow here the customary description, in which the cochlea is placed in such a manner that the base is directed downward, the summit upward; accordingly, "inner" is toward the axis of the cochlea, "outer" toward the periphery.

cate continuation of the periosteum which clothes both scalæ. On the outer wall of the cochlea the periosteum becomes greatly thickened, and in cross-section appears as a crescentic mass, the *ligamentum spirale*, which extends both above and below the attached surface of the ductus cochlearis.

The structure of the outer and the vestibular wall of the membranous cochlea is comparatively simple, that of the tympanic wall, on the other hand, is extremely complicated.

The outer wall and the spiral ligament together consist of epithelium and connective tissue. The latter, next to the bone, is a dense fibrous tissue; this passes into a loose connective tissue which contributes the chief bulk of the spiral ligament. The epithelium is composed of a row of cubical epithelial cells. A dense network of blood-vessels, the *stria vascularis*, occupies three-fourths of the height of the outer cochlear wall. At its lower end a vein

FIG. 243.—SECTION THROUGH THE SECOND TURN OF THE COCHLEA OF AN INFANT. $\times 25$. The modiolus contains longitudinal canals cut obliquely. *s.* Bony wall between the second and third (half) turns of the cochlea. The membrana vestibularis is torn, the upper fragment being turned upwards. The membrana tectoria can not be seen. Techn. No. 186.

projects into the lumen of the cochlea, the *prominentia spiralis* (vas prominens) (Fig. 243). The capillaries of the stria vascularis lie close beneath the epithelium; they are the source of the endolymph.

The *vestibular wall* (*Reissner's membrane*), consists of a process of the periosteum of the scala vestibuli, that is of delicate fibrous tissue and flattened cells, which on the surface turned toward the ductus cochlearis is clothed with a simple layer of polygonal epithelial cells.

The *tympanic wall* consists of two portions: the *limbus*, with the free margin of the bony spiral lamina, and the *lamina spiralis membranacea*.

The *limbus* consists of compact connective tissue, containing an abundance of spindle-shaped cells, which below is continuous with the tissue of the periosteum, and on its free surface is beset with peculiarly-shaped papillæ.

They have the form of an irregular hemisphere; toward the labium vestibulare they become small elongated plates, the so-called *auditory teeth*, which lie in a single row next to one another. The surface of the limbus is covered by a simple layer of flattened epithelial cells, which at the edge of the labium vestibulare passes into the cubical epithelium of the sulcus spiralis (Fig. 247, *A*).

FIG. 244.—A SURFACE VIEW OF LAMINA SPIRALIS OF CAT. $\times 240$. The vestibular lamina is seen from above. Between the auditory teeth two nuclei of the epithelial cells are visible. On the left of the picture the upper surface of the auditory teeth is in focus, on the right, the plane of the zona perforata. Techn. No. 185.

The upper surface of the free margin of the osseous spiral lamina is perforated by a single row of slit-like openings, the *foramina nervina*, through which the nerves enclosed within the bony lamina emerge, to penetrate within the epithelium of the basilar membrane. This portion of the osseous spiral lamina is called *zona perforata*.

The *membranous spiral lamina* comprises: (1) the *membrana basilaris*, an extension of the limbus and of the periosteum of the osseous spiral lamina; (2) the *tympanic lamella*, which is a process of the periosteum of the scala tympani and clothes the lower surface of the basilar membrane; and (3) the *epithelium* of the *ductus cochlearis*, which rests upon the upper surface of the basilar membrane.

The *membrana basilaris* consists of a structureless substance which contains rigid, perfectly straight fibers, extending from the labium tympanicum to the spiral ligament, and also oblong nuclei. The membrane has a finely striated appearance (Fig. 245, *f*).

The *tympanic lamella* is composed of a delicate connective tissue containing spindle-cells, the fibers of which are disposed vertically to the elements of the basilar membrane (Fig. 245, *b*).

The *epithelium* of that half of the membranous spiral lamina toward the axis of the cochlea is differentiated as the highly-specialized neuro-epithelium, the *spiral organ* (organ of Corti), while that occupying the outer half, toward the spiral ligament, consists of indifferent epithelial elements. The spiral lamina is therefore divided into two zones: an inner, occupied by the spiral organ, *zona tecta*—and an outer, *zona pectinata*—so called because of the striations of the basilar membrane shimmering through it.

The most remarkable elements of the spiral organ are the *pillar-cells* or rods of Corti, peculiarly-shaped and for the greater part rigid forms, arranged

FIG. 245.—SURFACE VIEW OF LAMINA SPIRALIS MEMBRANACEA OF CAT. $\times 240$. Layers of the zona pectinata drawn with change of focus. *e*, Indifferent epithelium (cells of Claudius) of the ductus cochlearis in focus; *f*, the fibers of the membrana basilaris in focus; *b*, the nuclei of the tympanic lamella in focus. Techn. No. 185.

in two rows throughout the entire length of the cochlea; an inner row, the *inner pillars*, and an outer row, the *outer pillars* (Fig. 247). The two rows of pillars converge and form an arch, the *arcus spiralis*, which spans a triangular

FIG. 246.—LAMINA SPIRALIS OF CAT SEEN FROM THE VESTIBULAR SURFACE. The membrana tectoria has been removed. $\times 50$. *lo.* Lamina spiralis ossea, inner half cleft and broken at several points. Cells of the spiral ganglion project from the posterior border of the same. *lm.* Lamina spiralis membranacea. The cells of Claudius have partly fallen off, so that the fibers of the membrana basilaris are visible as a delicate striation. Techn. No. 185.

FIG. 247.—SCHEME OF THE STRUCTURE OF THE TYMPANIC WALL OF THE DUCT OF THE COCHLEA. *A.* Seen from the side. *B.* Seen from the surface. In the latter, the free upper surface is in focus. It is evident that the epithelium of the sulcus spiralis, lying in other planes, as well as the cells of Claudius, can only be distinctly shown by depressing the tube. The membrana tectoria has not been drawn. The spiral nerve-fibers are indicated by dots.

space, the *tunnel*, the base of which is directed toward the basilar membrane. The tunnel is nothing more than a very large intercellular space, filled with a soft mass, the intercellular substance.

Regarding the histology of the pillar-cells, the following details are to be considered: The *inner pillar-cells* are rigid bands in which a *three-sided expanded base*, a *slender body*, and *concave head*, with the concavity directed outward, may be distinguished. The head is furnished with a thin process, the "head-plate" (Fig. 247). The body and base of the cell are surrounded by a scant amount of protoplasm, which only to the outer side of the base, in the vicinity of the nucleus, is present in somewhat larger amount. The *outer pillar-cells* exhibit the same details, excepting that the portion containing the nucleus lies to the inner side of the base; the rounded articular head rests in the concave facet of the head of the inner pillar-cells; the broader head-plate is covered in its greater part by the head-plate of the inner pillars. To the inner side of the inner pillars lies a simple row of cells, the *inner hair-cells*, short cylindrical elements that do not extend to the basilar membrane; they possess a rounded base and about twenty stiff hairs on their free surface. To the inner side of the inner hair-cells lies the cubical epithelium of the sulcus spiralis. On

FIG. 248.—SURFACE VIEW OF LAMINA SPIRALIS MEMBRANACEA OF CAT. $\times 240$. *A*. Outer pillar-cells: *k*, head-plates of the same, upper surface in focus; *ap*, body and inferior extremity drawn with gradual depression of the tube; *kip*, portions of the head-plates of the inner pillar-cells. *B*. *tt*, Labium tympanicum partly covered by the epithelium of the sulcus spiralis; *ih*, inner, *ah*, outer hair-cells, between these the phalanges *ph*, forming the membrana reticularis; *ap*, head-plates of the outer, *ip*, of the inner pillar-cells. Techn. No. 185.

the outer side of the outer pillars lie the *outer hair-cells*; they resemble the inner hair-cells, but are characterized by a dark body occupying the upper half of the cell, the *spiral body*.* The outer hair-cells are arranged in several (usually four) rows; they do not lie in contact with one another, but are held apart by *Deiters's cells*; these are elongated cells that contain a rigid filament and possess at their upper ends a *cuticular end-plate*; this has the form of a digital phalanx. The free spaces between the "phalanges" are occupied by the upper ends of the outer hair-cells (Fig. 248). The cells of Deiters are sustentacular elements that exhibit much in common with the pillar-cells; like these they consist of a rigid filament and a protoplasmic portion; like these they have a head-plate (named phalanx). The difference consists only in this, that the transformation into rigid parts is not so far advanced. The phalanges are joined to one another and form a beautiful netted membrane, the *membrana reticularis*.

* In the scheme (Fig. 247, *A*) this body is indicated by a dark dot close beneath the auditory hairs.

The outer hair-cells do not extend to the basilar membrane, but occupy only the upper half of the spaces between the cells of Deiters; the lower divisions of these spaces remain unoccupied, and are called *Nuel's spaces* (or, since they communicate with one another, the space of Nuel), which are inter-cellular clefts, like the tunnel, with which they connect.

External to the last row of Deiters's cells lie the cells of Heusen, elongated cylinders, that gradually decrease in height and pass into the indifferent epithelium of the duct of the cochlea, whose elements over the remaining part of the basilar membrane are called the *cells of Claudius*.

A soft, elastic cuticular formation, the *membrana tectoria*, extends over the sulcus spiralis and the organ of Corti (Fig. 249). It is attached to the vestibular lip of the sulcus and extends to the outermost row of hair-cells.

FIG. 249.—VERTICAL RADIAL SECTION THROUGH THE PERIPHERAL HALF OF LAMINA SPIRALIS OSSEA AND THROUGH THE LAMINA SPIRALIS MEMBRANACEA OF AN INFANT. $\times 80$. The membrana tectoria has been torn off from its point of attachment to the labium vestibulare. Techn. No. 186.

The *cochlear branch* of the *auditory nerve* penetrates into the axis of the cochlea and in its spiral uninterrupted course gives off branches which pass to the root of the osseous spiral lamina; here each medullated nerve-fiber loses its medullated sheath and passes into a nerve-cell which like those of the spinal ganglia possesses a connective-tissue capsule; these nerve-cells collectively form the *ganglion spirale*,* which winds along the entire peripheral spiral canal of the cochlea (Fig. 243). From the opposite pole of each cell springs a second nerve-fiber, that soon acquires a medullated sheath and unites with neighboring fibers in a wide-meshed plexus enclosed within the osseous spiral lamina; it extends near to the labium tympanicum, where the fibers lose their medullated sheath, escape through the foramina nervina, and end in the epithelium in the following manner: they bend in the direction of the turns of the cochlea and run in spiral bundles, of which the first passes to the inner side of the inner pillar-cells, the second to the tunnel, the third between the outer

* The *ganglion spirale* possesses the same structure as the spinal ganglia, with a single difference,—the ganglion-cells are not unipolar, but bipolar, as in the embryonal ganglia.

pillar-cells and the first row of the cells of Deiters, the remaining three between the cells of Deiters. From these bundles delicate fibers proceed to the hair-cells, on which (not within) they terminate.

The *arteries* of the labyrinth come from the auditory and the stylomastoid artery, which send a branch through the fenestra rotunda to the cochlea. The auditory artery sends branches to the saccules and to the semicircular canals, which in general supply a wide-meshed capillary network, but a close-meshed network on the maculæ and cristæ; and a branch to the cochlea, which on entering the same breaks up into a number of small branches. These in part enter the first turn, in part ascend in the axis of the cochlea. From the latter branches small twigs diverge successively and enter the bony wall of the modiolus, where they form the radicles of smaller and larger masses of coiled blood-vessels, the *glomeruli cochleæ minores et majores*. The smaller glomeruli are situated somewhat above the point of origin of the osseous spiral lamina and supply capillaries to the limbus and to the vestibular membrane. The larger glomeruli lie at the root of the septum between the adjoining turns of the cochlea and supply two independent vascular territories—the stria vascularis and the lamina spiralis membranacea. The *veins* unite in the vas prominens and in the vas spirale, which empty into the vena spiralis modioli lying beneath the ganglion spirale within the modiolus. The latter probably empties through the aquæductus cochleæ into the internal jugular vein.

The arrangement of the blood-vessels of the cochlea is such that the scala vestibuli is encircled by arteries, the scala tympani by veins. The upper portion of the scala tympani bounding the membranous spiral lamina is thus completely removed from the influence of arterial pulsation.

The Lymph-channels.—The endolymph in the interior of the membranous labyrinth communicates with the subdural lymph-spaces by means of minute tubules passing from the base of the ductus endolymphaticus. The perilymphatic spaces connect with the subarachnoidal spaces by means of the “ductus perilymphaticus,” a lymph-vessel running through the aquæductus cochleæ.

THE MIDDLE EAR.

The *mucous membrane of the tympanic cavity* is intimately united with the underlying periosteum. It consists of a thin connective-tissue tunica propria and a single stratum of cubical epithelial cells, that sometimes on the floor, occasionally also in larger areas of the tympanic cavity, is ciliated. Glands (short, 0.1 mm. long follicles) occur sparingly in the anterior half of the tympanic cavity. The *mucosa of the Eustachian tube* consists of a fibrous tunica propria (containing numerous leucocytes near the pharyngeal orifice) and of a stratified ciliated columnar epithelium; the ciliary wave is directed toward the pharynx. Mucous glands occur in especial abundance in the pharyngeal half of the tube. The cartilage of the Eustachian tube, where it adjoins the bony tube, is of the hyaline variety, and here and there contains rigid (not elastic) fibers; in the anterior portion the matrix is penetrated by dense networks of

elastic fibers. The *blood-vessels* in the mucosa of the tympanic cavity form a wide-meshed, in the mucosa of the Eustachian tube a narrow-meshed superficial capillary network, and a deep capillary plexus surrounding the glands. The *lymph-vessels* run in the periosteum of the tympanic cavity. With regard to the terminations of the nerves, exact information is still wanting.

THE EXTERNAL EAR.

The *tympanum* consists of a lamina of connective tissue, *lamina propria*, in which the fibrous bundles on the outer surface are radially arranged and connected with the periosteum of the sulcus tympanicus; while on the inner surface, toward the tympanic cavity, they are circularly arranged. On its inner surface the membrane tympani is covered by the mucous membrane of the tympanic cavity, on its outer surface by the integument of the external auditory canal. Both investments are very firmly attached to the lamina propria, are smooth, and are without papillae. Where the malleus lies against the tympanum, the latter is provided with a superficial stratum of hyaline cartilage.

The *external auditory canal*, as far as it is cartilaginous and on the whole length of its upper wall, is clothed with an extension of the skin characterized by its thickness and by a great abundance of peculiar coil-glands, the *ceruminous glands*. In some respects these glands correspond with the ordinary

larger coil-glands (sweat-glands) of the skin; like these, they possess an excretory duct, lined by several layers of epithelial cells, and the tubules of the coil contain a simple layer of cubical gland-cells, resting on smooth muscle-

FIG. 250.—FROM A VERTICAL SECTION THROUGH THE SKIN OF THE EXTERNAL AUDITORY MEATUS OF AN INFANT. $\times 50$. The excretory duct opens into the hair-follicle. Techn. No. 189.

FIG. 251.—A. CROSS-SECTION OF THE COIL-TUBULE FROM THE SAME PREPARATION (Fig. 250). B. LONGITUDINAL SECTION OF A COIL-TUBULE FROM THE EXTERNAL AUDITORY MEATUS OF A TWELVE-YEAR-OLD BOY. $\times 240$. Techn. No. 189.

fibers and a conspicuous basement membrane; they are distinguished from the sweat-glands by the very wide lumen of the coiled tubule, that, especially in adults, is greatly dilated, and by numerous pigment-granules and fat-droplets within the gland-cells, which frequently exhibit a distinct cuticular border.

The excretory ducts are narrow, and in children open in the hair-follicles; in adults, close beside the hair-follicles on the free surface. The secretion, the *cerumen*, consists of pigment-granules, oil-globules, and cells containing fat; the latter probably come from the sebaceous glands. In the (remaining) region of the bony external auditory meatus, the integument is thin and without ceruminous glands.

The cartilage of the external auditory canal and of the pinna is of the yellow elastic variety.

The *blood-vessels* and *nerves* are distributed as in the skin elsewhere; only on the tympanum do they exhibit peculiarities. Along the handle of the malleus an artery descends, which breaks up into radially-disposed branches; the blood is returned by a vein that, likewise, runs along the handle of the malleus. The vessels lie in the integumentary covering of the tympanum. The mucous membrane of the tympanum is provided with a dense capillary network, which anastomoses with the integumentary vascular network by means of perforating branches.

The *lymph-vessels* are found principally in the cutaneous stratum of the tympanum.

The *nerves* form delicate networks beneath both the mucous and the cutaneous layers.

XII. THE NASAL MUCOUS MEMBRANE.

The nasal mucous membrane is composed of three divisions differing in structure: that of the *vestibular region*, that of the *respiratory region*, and that of the *olfactory region*.

THE VESTIBULAR REGION.

The mucous membrane of the vestibular region (that lining the movable nose) is a modified continuation of the integument and consists of a tunica propria beset with papillæ and covered by a stratified squamous epithelium. Numerous sebaceous glands and the hair-follicles of the stiff nasal hairs (*vibrissæ*) are embedded in the tunica propria.

THE RESPIRATORY REGION.

The respiratory region of the nasal mucous membrane in man includes that lining all parts of the nasal fossæ (and the accessory nasal spaces), except that upon the median portion of the superior turbinal and the corresponding part of the nasal septum. It consists of a *stratified ciliated epithelium*, sometimes containing few goblet-cells, sometimes many, and of a conspicuous *tunica propria*, 4 mm. thick on the inferior turbinal, which is composed of

fibrillar connective tissue and a large, variable number of leucocytes; occasionally the latter form solitary nodules. Migration of leucocytes through the epithelium into the nasal fossæ also occurs.

The tunica propria in man contains branched tubular glands, which produce both mucous and serous secretion, and are therefore mixed glands. Not infrequently they open in funnel-shaped depressions, which are lined by an extension of the superficial epithelium, and on the inferior turbinal are perceptible by the unaided eye.

In the accessory nasal spaces the epithelium and tunica propria are considerably thinner (~ 0.02 mm.), but otherwise of the same structure; the glands are small and few in number.

THE OLFACTORY REGION.

The olfactory division of the nasal mucous membrane in man is limited to the median portion of the superior turbinal and the corresponding part of the

FIG. 252.—THICK VERTICAL SECTION OF RESPIRATORY MUCOUS MEMBRANE OF HUMAN NASAL SEPTUM. $\times 20$. The excretory ducts of two glands are visible. *f*, Funnel-shaped depression; *v*, vein. Techn. No. 191.

nasal septum, and is distinguished, macroscopically, from the rosy mucosa of the respiratory division by its yellowish-brown color. It consists of an epithelium, the olfactory epithelium, and of a tunica propria. In the olfactory epithelium, two forms of cells occur. The one form is cylindrical in its upper half and contains a yellowish pigment and minute granules, often arranged in longitudinal rows. The lower half is slenderer, the edge is serrated and indented, the inferior end is forked, and is said to unite with the similar ends of neighboring cells to form a protoplasmic network. These elements are called *sustentacular cells*. Their nuclei are usually oval and lie at the same level; in vertical sections they are seen to occupy a narrow belt, the *zone of the oval nuclei* (Fig. 255). The second form of cells possesses a spherical nucleus and only in the vicinity of the latter an appreciable amount of protoplasm; from this a slender ciliated cylinder, the attenuated cell-body, extends upward, while from the opposite pole a very delicate process continues directly into the axis-cylinder of a nerve-fiber. These cells, the *olfactory cells*, are ganglion-cells, and their lower process a centripetal nerve-fiber. Their round nucleolated nuclei

lie at different levels and occupy a broad belt, the *zone of the round nuclei*. Occasionally, in the nonnucleated epithelial territory, round nuclei in varying number are found above the zone of the oval nuclei; they belong to dislocated olfactory cells, or are the nuclei of wandering, often pigmented, leucocytes. In addition to these two kinds of cells, there are intermediate forms, which sometimes resemble the olfactory elements, sometimes the sustentacular cells. At the border of the epithelium, toward the connective tissue, is a protoplasmic network furnished with nuclei, the so-called *basal cells* (Fig. 256, *b*). The surface of the epithelium is covered by an extremely delicate homogeneous membrane, the *membrana limitans olfactoria*; it is pierced by the ciliated extremities of the olfactory cells and is covered by a peculiar substance, regarded by some authors as a cuticular formation similar to the basal border of the intestinal epithelium, by others as delicate cilia, by still others interpreted as minute particles of discharged mucus (Fig. 253, *s*).

The *tunica propria* consists of a loose feltwork of rigid connective-tissue fibers intermingled with delicate elastic fibers, which in some animals toward the epithelium (for example, in the cat) is condensed to a structureless membrane. Numerous glands, the so-called *olfactory* (*Boreman's*) *glands*, are em-

FIG. 253.—ISOLATED CELLS OF THE OLFACTORY MUCOSA OF RABBIT. $\times 560$. *st*, Sustentacular cells; *s*, extruded mucus resembling cilia; *r*, olfactory cells, at *r'* the lower process has been torn off; *f*, ciliated cells; *b*, cells of olfactory glands. Techn. No. 190.

FIG. 254.—VERTICAL SECTION THROUGH THE OLFACTORY REGION OF A YOUNG RAT. $\times 480$. Techn. No. 193

bedded in the tunica propria; they are either simple or (for example, in man) branched tubules, in which an excretory duct, situated in the epithelium, a body and a fundus may be distinguished. The cells of the body of the glands are pigmented. The glands were until recently regarded as serous glands, but latterly they have been pronounced mucous glands. The olfactory glands fre-

quently advance beyond the territory of the olfactory mucous membrane, and are found in the adjoining portions of the respiratory mucous membrane. The tunica propria also carries the ramifications of the nerves. The branches of the olfactory nerve are accompanied by processes of the dura and consist

FIG. 255.—VERTICAL SECTION OF OLFACTORY MUCOSA OF RABBIT. $\times 50$. *zo*, Zone of oval nuclei; *zr*, zone of round nuclei; *dr*, olfactory glands; *a*, excretory duct; *k*, body; *g*, fundus; *n*, branches of olfactory nerve cut transversely; *v*, veins; *ar*, arteries; *b*, bundles of connective tissue in cross-section. Techn. No. 192.

throughout of nonmedullated fibers, that readily separate into their component fibrillæ; the fibers are the inferior processes of the olfactory cells, grouped in bundles, which pass in horizontal arches from the epithelium, descend into

FIG. 256.—VERTICAL SECTION THROUGH THE OLFACTORY MUCOSA OF RABBIT. $\times 560$. *s*, Cuticular border; *zo*, zone of oval nuclei; *zr*, zone of round nuclei; *b*, basal cells; *dr*, portions of olfactory glands, on the right the lower portion of the excretory duct is visible; *n*, branch of the olfactory nerve. Techn. No. 192.

the tunica propria, and by union with neighboring bundles form the branches of the olfactory nerve. The terminal ramifications of the trifacial nerve lie within the tunica propria; delicate fibers that ascend to the epithelium and there terminate in free ends possibly belong to the trifacial nerve.

Of the *blood-vessels* of the nasal mucosa the stems of the arteries run in the deeper strata of the tunica propria; they break up into a rich subepithelial capillary network. The *veins* are remarkable for their size, and over the posterior end of the inferior turbinal form so dense a network as to give the tunica propria the character of cavernous tissue (Fig. 252 and Fig. 255).

The *lymph-vessels* form a coarse-meshed net lying in the deeper strata of the tunica propria. The lymph-vessels of the olfactory mucosa may be injected from the subarachnoid space, through the perineurial sheaths of the branches of the olfactory nerve, acquired from the cerebral membranes on passing through the cribriform plate.

Medullated twigs of the trifacial nerve may be found in the respiratory as well as in the olfactory mucosa.

XIII. THE TASTE-BUDS.

The *taste-buds* or *gustatory organs* are oval bodies, about $80\ \mu$ long and $40\ \mu$ broad, which are completely embedded in the epithelium of the oral mucous membrane; their base rests upon the tunica propria, the upper end reaches to the surface of the epithelium, which at this point exhibits a funnel-shaped de-

FIG. 257.—VERTICAL SECTION OF TWO RIDGES OF PAPILLA FOLIATA OF RABBIT. $\times 80$. Each ridge, *l*, bears secondary ridges, *l'*; *g*, taste-buds; *n*, medullated nerves; *d*, serous gland; *a*, portion of an excretory duct of a serous gland; *m*, muscle-fibers of tongue. Techn. No. 195.

pression, the *taste-pore*. Each taste-bud consists of two kinds of elongated epithelial cells; the one are either of the same diameter throughout, or they taper at the basal end, which occasionally is forked, while the upper end is prolonged to a fine pointed extremity; their protoplasm is clear. These cells constitute the bulk of the taste-bud, are principally situated at the periphery, and are called

tegmental cells. They serve as support and sheath for the *gustatory cells*, which are the real percipient epithelial elements. The gustatory cells are small, and only slightly enlarged where the nucleus is situated; the latter is sometimes near the lower or central end, sometimes in the middle, rarely at the upper or peripheral end of the cell. The upper division of the cell is cylindrical, or more frequently conical, and bears on its free end a stiff, refractile, hair-like process, a cuticular formation (Fig. 258); the lower division is sometimes slender, sometimes thick and blunted at the end or expanded into a triangular

FIG. 258.—FROM A VERTICAL SECTION OF PAPILLA FOLIATA OF RABBIT. $\times 560$. Techn. No. 195.

foot, which does not however extend into the fibrous tissue of the mucosa. Their protoplasm is granular. Not infrequently many leucocytes are found in the interior of the taste-bud.

The taste-buds occur chiefly in the lateral walls of the circumvallate papillæ and on the ridges of the papillæ foliatæ, also occasionally on the papillæ fungiformes, on the soft palate, and on the posterior surface of the epiglottis.

FIG. 259.—FROM A VERTICAL SECTION OF A CIRCUMVALLATE PAPILLA OF MONKEY. $\times 240$. Techn. No. 196.

The conjecture that the terminal ramifications of the glossopharyngeal nerve have the same anatomical relation to the gustatory cells that the olfactory nerve-fibers have to the olfactory cells has been shown to be erroneous. The terminal branches of the glossopharyngeal nerve consist of medullated and gray nerve-fibers beset with microscopic (sympathetic) ganglia,* which form a dense plexus in the tunica propria, from which numerous branches spring.

* Whether the so-called "taste-granules" beneath the epithelium of the papillæ foliatæ are multipolar nerve-cells is very questionable; a nerve-process has not as yet been demonstrated.

Some of the latter terminate, possibly, in the connective tissue in end-bulbs, but the majority of the gray fibers penetrate into the epithelium. Here two kinds of fibers may be distinguished. The one kind, the "intragemmal" fibers, enter the taste-buds, divide, and form a plexus beset with numerous conspicuous varicosities that extends up to the taste-pore; these fibers do not anastomose with one another, nor do they connect with the gustatory cells, but all terminate in free ends. The other, the smoother "intergemmal" fibers, penetrate the epithelial areas between the taste-buds and, usually without dividing, extend to the uppermost strata of the epithelium.

PART III.

SPECIAL TECHNIQUE.

I. KARYOKINESIS.

NO. 1.—For the study of nuclear structure and karyokinesis amphibian larvæ are most suitable. Those most readily procured are the larvæ of the water salamander, which in the months of June and July abound in every pool. Place freshly-caught specimens, 3 to 4 cm. long, in about 100 c.c. of chromo-acetic acid (p. 21). After 3 hours place the larvæ in running water for 8 hours, and then in 70 per cent. alcohol. At the expiration of 4 hours, or later, the objects are ready for further treatment.

a. Nuclear Structure.—With a scalpel carefully scrape the epithelium from the skin of the abdomen, with two pairs of delicate forceps strip off the thin corium, stain it 1 to 3 minutes in 5 c.c. of Böhmer's hematoxylin (p. 31), and mount in damar-varnish (p. 38). Between the round glands beautiful connective-tissue cells with large nuclei may be seen. The structure of the protoplasm, the centrosome and attraction-sphere, also the structure of the nucleus can only be recognized by the employment of complicated methods and high magnification. The results obtained by ordinary methods are like that pictured in Fig. 260.

FIG. 260.—CONNECTIVE-TISSUE CELL FROM CORIUM OF TRITON TENIATUS, SURFACE VIEW. $\times 560$. Only the coarser filaments of the nuclear network can be distinctly seen; with this magnification the finer filaments appear as minute dots, the nucleoli as parts of the nuclear network.

The cross-striped muscles of the tail and the membranes of smooth muscle-fiber (the latter may be readily obtained by stripping off the muscularis of the intestine) also furnish instructive slides.

b. Karyokinesis.—With a pair of fine scissors cut round the margin of the cornea, and strip off the same; stain and preserve like *a*. The preparation must be placed on the slide with the convex surface of the cornea upward; in the epithelium, even with the low-power objective, many karyomitotic figures may be seen, which may be recognized by their intense color. By this method the nuclear spindle and polar radiation, as in Fig. 5, can only be perceived (with higher magnification) in especially favorable preparations, *e. g.*, eggs of sireon and the trout.

The delicate lamellæ suspended from the convex side of the cartilaginous-gill-arch, as well as the epithelium of the floor of the oral cavity, are very suitable objects. Occasionally not a single karyokinetic figure is found. Isolated figures may sometimes be observed in preparation *a*.

II. CILIATED EPITHELIAL CELLS.

No. 2.—To obtain living ciliated cells, kill a frog (p. 25), place it on its back and with scissors cut off the lower jaw, so that the roof of the cavity of the mouth is exposed. From the mucosa of the roof cut out a small strip about 5 mm. long, place it on the slide in a drop of salt solution, and apply a cover-glass. Examine with the high power and search the edges of the preparation. At first the movement of the cilia is very lively, so that the observer cannot see the individual cilia: the entire ciliated border waves; the motion has been compared to a cornfield swayed by the wind. After a few moments the rapidity of the movement diminishes and the cilia can be plainly seen. If the movement ceases, it can be restored by the application of a drop of concentrated potash solution (p. 41); the effect is transient, so that the eye of the observer must not be removed from the ocular while the fluid passes under the cover-glass. The addition of water soon suspends the movement.

III. CONNECTIVE TISSUE.

No. 3.—*Mucous Connective Tissue*.—Place the umbilical cord of a 3 to 4 months' human embryo (or pig embryo 3 to 6 cm. long) in 100 c.c. of Müller's fluid (p. 20) 3 to 4 weeks; harden in 30 c.c. of gradually strengthened alcohols (p. 29). The cord will still be very soft; in order to obtain good sections it must be embedded in liver, and in cutting must be somewhat compressed with the fingers. The section may be stained in picrocarmine (12 hours) or in hematoxylin (5 minutes), and should be examined in a drop of distilled water. In glycerine and damar-varnish the delicate processes of the cells and the bundles of connective tissue are invisible. In the vicinity of the blood-vessels the network of cells is less fine; therefore a field remote from the blood-vessels should be selected for study. The older the embryo, the greater is the number of the connective-tissue bundles. Mount in diluted glycerine (p. 21).

No. 4.—*Fibrous Connective Tissue: Connective-tissue Bundles*.—Prepare small strips, 1 to 2 cm. long, of intermuscular connective tissue, for example, of the thin septum between the serratus and intercostal muscles; place a small piece on a dry slide and quickly spread it out with teasing needles (see "half-drying method," No. 27 *a*, p. 281), add a drop of salt solution and apply a cover-glass. The bundles of connective tissue appear wavy and pale: with a little practice the sharply-contoured, highly-refracting elastic fibers may be distinguished, and also, in favorable situations, the nuclei of the connective-tissue cells.

No. 5.—The *cells of fibrous connective tissue* may be rendered visible by the addition of a drop of picrocarmine to preparation No. 4, under the cover-glass (p. 41). In most cases only the red nucleus can be perceived, especially when the cell lies wholly upon the fibrous bundles. In rare cases the pale yellow, variously-shaped body of the cell can be seen (Fig. 22, *A*, 1, 2, 3).

No. 6.—*Mastzellen* (granule-cells).—Fix small pieces, 1 to 2 cm. square, of mucous membrane (of the mouth, of the pharynx, or of the intestine) in absolute alcohol (p. 27). In from 3 to 8 days cut thin sections and stain them in 10 c.c. of alum-carmine dahlia for 24 hours (p. 23). Transfer them to 10 c.c. of absolute alcohol for 24 hours, which must be renewed once or twice during this time. Mount in damar (p. 38). The protoplasm of the Mastzellen then exhibits granules stained an intense blue.

No. 7.—*Fibrillæ*.—Place a piece of tendon about 2 cm. long in a saturated aqueous solution of picric acid. On the following day, with two pairs of forceps, pull the tendon apart along its length, take from the interior a bundle about 5 mm. long, and tease the same on a dry slide (*cf.* No. 27 *a*, p. 281), add a drop of distilled water, apply a cover-glass, and examine with the high-power objective. The ultimate fibrillæ appear as exceedingly fine, silky filaments.

No. 8.—“*Encircling Fibers*.”—With the scissors cut out a piece about 1 cm. square of the connective tissue within the arterial circle of Willis, wash it in a watch-glass in salt solution, with needles spread it out in a drop of the same solution on a slide, and cover. With the low power, in addition to numerous delicate blood-vessels and ordinary bundles of fibrous tissue, sharply-contoured, refracting bundles, in distinct contrast to the remaining connective tissue, will be found, which, on the use of the high power and a diaphragm of narrow aperture, show that they, likewise, consist of fibrillar connective tissue. Place such a bundle in the field and treat it with a drop of acetic acid under the cover-glass (p. 41). As soon as the acid reaches the bundle, it swells, the fibrillation vanishes, and instead elongated nuclei appear. The swelling is not uniform; at irregular intervals the bundle is constricted. With dim illumination the “fibers” (cell remnants) producing the constrictions may be seen (Fig. 22, *B*).

No. 9.—*Fat-cells*.—Take a small piece of the reddish-yellow, gelatinous fat from the axilla of an emaciated individual; spread out *rapidly* a piece the size of a split pea in the *thinnest possible* layer on a dry slide, add *immediately* a drop of salt solution, and apply a cover-glass. In thin places atrophic fat-cells, like those shown in Fig. 23 *B*, will be seen. This preparation may be stained under the cover-glass with picrocarmine (p. 41) and preserved in diluted glycerine. Ordinary (normal) fat-cells, taken from any part of the body, are likewise to be examined in salt solution. The spherical cells should be studied with change of focus (*cf.* Fig. 23, *A*).

No. 10.—*Fine elastic fibers* may be readily obtained by treating preparation No. 4, under the cover-glass, with a few drops of acetic acid. The connective-tissue bundles swell and become transparent, the elastic fibers, on the contrary, remain unaltered, and stand out sharply contoured (Fig. 20, *A*).

No. 11.—*Thicker elastic fibers* may be obtained by teasing in a drop of salt solution a slender piece, about 5 mm. long, of the fresh ligamentum nuchæ of an ox (Fig. 20, *B*). The piece should not be taken from the loose, enveloping tissue, but from the tough, yellowish fibrous portion. The preparation may be stained in picrocarmine and mounted in glycerine.

No. 12.—*Cross-sections of thick elastic fibers* may be obtained by drying a piece (10 cm. long and 1 to 2 cm. thick) of the ligamentum nuchæ (it will be ready to use in 4 to 6 days) and treating it like No. 63.

No. 13.—*Fenestrated Membranes*.—Take a small piece (about 5 mm. square) of endocardium, place it in a drop of water on a slide, and add, under the cover-glass, 1 to 2 drops of potash-lye. Examine the edges of the preparation (Fig. 21).

Good specimens may also be obtained from the basilar artery; place a piece of the artery cut open lengthwise in 10 c.c. of concentrated potash solution. After 6 hours take a small piece, about 1 cm. long, and separate the lamellæ in a drop of water on a slide; this is easily done by scraping it with a scalpel. Cover and examine with the high power. The small apertures in the membrane have the appearance of shining nuclei.

With the low power the membrane is to be recognized by its dark outlines. To preserve, wash it well in 10 c.c. of water (5 minutes), stain it in 3 c.c. of Congo red from 12 to 20 hours (p. 23), and mount in damar.

No. 14.—*Hyaline Cartilage*.—Cut off the extremely thin episternum of the frog, place it on a dry slide, cover it with a cover-glass, and examine at once with the high power. The cartilage-cells completely fill the lacunæ (Fig. 25, *A*). For prolonged study, add a drop of saline solution.

No. 15.—*Hyaline Costal Cartilage*.—Without any previous preparation fine sections of costal cartilage may be cut with a dry razor, and examined in a drop of water. Search for one of the glossy areas containing rigid fibers (Fig. 25, *B*). The preparation may be preserved by adding a few drops of dilute glycerine.

Fresh cartilage does not stain readily. The tissue must first be placed in Kleinenberg's picrosulphuric-acid mixture or in Müller's fluid and then in alcohol (p. 29) and subsequently stained with Böhmer's hematoxylin (p. 22). Mounted in damar, which clears vigorously, the finer details vanish.

No. 16.—*Elastic Cartilage*.—Take a piece of the arytenoid cartilage of man (better still of the ox)—the elastic cartilage of the anterior angle is recognized by its yellowish color. Cut a section that includes the boundary line between the elastic and hyaline cartilage, and examine it in water. Preserve like No. 15. The development of the elastic fibers may often be studied in the cartilages of adults, especially in the epiglottis and in the vocal process of the arytenoid cartilage (Fig. 26, 1).

No. 17.—*White Fibro-cartilage*.—Cut the intervertebral disks of adult man in pieces from 1 to 2 cm. square; fix in 100 c.c. of picrosulphuric acid (p. 20). for 24 hours and harden in 50 c.c. of gradually strengthened alcohols (p. 29). Stain sections in Böhmer's hematoxylin (p. 22) and mount in damar (p. 38). Sections through the edges yield hyaline cartilage; sections through the central portions of the disk exhibit large groups of cartilage-cells.

IV. MUSCLE-FIBERS.

No. 18.—*Striated Muscle-fibers*.—*a (of the frog)*.—With the scissors placed flat and parallel to the course of the fibers, cut a piece about 1 cm. long from the adductor muscle of a recently-killed frog. Take a fragment from the inner surface of this piece and tease it in a small drop of salt solution, add a second larger drop of the same liquid and, *without pressing*, cover the preparation with a cover-glass. With low magnification (50 diameters) the cylindrical form, the difference in thickness, occasionally also the cross-striation of the isolated fibers may be seen (Fig. 34). With higher magnification (240 diameters) the cross-striation is distinctly visible, and occasionally pale nuclei and refracting granules. The presence of numerous granules within the muscle-fibers is probably an indication of active metabolic processes. Where the muscle-fibers are cut across, the muscle-substance not infrequently protrudes from the sarcolemma.

b (of man).—I have found beautiful striated fibers in muscles taken from the human cadaver injected with carbolic acid.

To preserve, stain under the cover-glass with picrocarmine (p. 41) for about 5 minutes, and then displace the staining fluid with diluted glycerine.

No. 19.—*The Sarcolemma*.—Treat preparation No. 18 *a* with a couple of drops of ordinary water. In 2 to 5 minutes it will be seen, with the low power (50 diameters), that the sarcolemma is raised from the muscle-substance in the

form of transparent blebs, and at other places, where the torn muscle-substance has retracted, the sheath appears as a delicate line spanning the interval (Fig. 34, 1, *s*, *s'*).

No. 20.—*Muscle Nuclei*.—Prepare muscle-fibers like No. 18 *a*. Treat with a drop of acetic acid (p. 41). The shrunken but sharply-outlined nuclei, with the lower power, have the appearance of spindle-shaped streaks (Fig. 34, 2).

No. 21.—*Fibrillæ*.—Place the fresh muscle of a frog in 20 c.c. of 0.1 per cent. chromic-acid solution (p. 20). In about 24 hours the tissue may be teased in a drop of water, and fibers will be found whose ends have separated into their ultimate fibrillæ (Fig. 34, 2). If it is desired to make a permanent preparation, place the muscle in water for 1 hour, then in 20 c.c. 33 per cent. alcohol, 10 to 20 hours; tease at once or preserve in 70 per cent. alcohol until wanted, and then isolate (p. 25). If the chromic acid be removed by allowing the tissue to remain in alcohol (frequently renewed) for several weeks, the teased preparation may then be stained with picrocarmine in the moist chamber and this replaced by glycerine (p. 41).

No. 22.—*The Ends of the Muscle-fibers*.—Place the fresh gastrocnemius muscle of the frog in 20 c.c. of concentrated potash-lye, and cover the watch-glass. In about 30 to 60 minutes (in a cold room, somewhat later) the muscle, if lightly moved with a glass rod, falls into its fibers. Should this fail, the solution is not strong enough (see p. 26). Transfer a number of the fibers in a drop of the same solution to a slide and carefully apply a cover-glass. With the low power the ends of the muscle-fibers and numerous nuclei may be seen (Fig. 34, 3). The fibers should not be examined in water or glycerine, since the lye, thus diluted, soon destroys them.

No. 23.—*Branched Muscle-fibers*.—Remove the tongue from a recently-killed frog (it is attached in front to the lower jaw, is free behind) and place it in 20 c.c. of pure nitric acid, to which about 5 gm. of potassium chlorate have been added (some undissolved chlorate must remain in the bottom of the vessel). In a few hours, with glass rods carefully transfer the tongue to 30 c.c. of distilled water, which must be frequently changed. In this the tissue can remain a week, though it may be used at the end of 24 hours. For this purpose put it in a test-tube half filled with water and shake it several minutes; the tongue will fall to pieces. Turn the contents of the test-tube into a capsule, and in an hour or later place a little of the sediment that has been deposited in the meanwhile in a drop of water on a slide. The tissue may be further isolated with the teasing needles, but in most cases this is superfluous. Examine with the low power. Stain under the cover-glass with picrocarmine (p. 41). Mount in dilute glycerine (p. 21). (Fig. 34, 4.)

No. 24.—*Smooth Muscle-fibers*.—These are best isolated by placing a piece of the stomach or intestine of a frog, just killed, in 20 c.c. of potash solution and treating like No. 22 (Fig. 31).

V. NERVE-CELLS AND NERVE-FIBERS.

No. 25.—*Ganglion-cells, Fresh*.—Tease a small piece of the Gasserian ganglion in a drop of salt solution, and stain under the cover-glass with picrocarmine for 2 minutes (p. 41). The processes of the cells usually tear off.

The ganglion-cells of the cerebral and cerebellar cortex may be prepared in the same way; the processes likewise are easily lost.

No. 26.—*Multipolar Ganglion of the Spinal Cord*.—Remove with the scissors as much as possible of the white substance of the spinal cord of an ox, and place the gray remnant in pieces 1 to 2 cm. in length in 30 c.c. of 33 per cent. alcohol (p. 19, 3, *d*). In 36 to 48 hours transfer the pieces to 20 c.c. of undiluted neutral carmine solution (p. 23) for 24 hours. The now very soft pieces should be transferred with the section-lifter to 50 c.c. of distilled water, in order to wash out some of the stain, and after 10 minutes spread with needles in a thin layer on a dry slide. The ganglion-cells can be distinguished by their bright red nuclei; the cell-body and the processes are not visible. Let the preparation dry thoroughly and mount in damar (Fig. 38).

No. 27.—*Fresh Medullated Nerve-fibers*.—Expose the sciatic nerve of a frog just killed, and with delicate scissors cut it at the level of the popliteal space and about 1 cm. higher. Isolate in a drop of salt solution.

No. 27 *a*.—Better still, tease on a dry slide by the "half-drying" method. Hold the *lower* end of the nerve with one needle, with another needle separate the nerve-bundles along half the length of the nerve; a thin shining membrane will span the interval between the separated bundles. Add a drop of salt solution and apply a cover-glass. The membrane contains numerous isolated nerve-fibers. The manipulation must be done very rapidly (in about 15 seconds), so that the nerve-fibers do not become dry (Fig. 41, 6, 7, 8, 9).

No. 28.—*Alterations in the Medullary Sheath*.—Treat No. 27 *a* with water (place a drop at the edge of the cover-glass and let it flow under). In a few minutes the formation of the myelin drops begins (Fig. 41, 10).

No. 29.—*The Axis-cylinder*.—Tease dry (like No. 27 *a*) and stain with methylene blue (p. 34); the nodes of Ranvier stain first, and often so deeply that the axis-cylinder cannot be recognized there. The axis-cylinder frequently shrinks and becomes displaced within the medullary sheath, or it contracts and becomes convoluted. On the addition of glycerine the medullary substance can no longer be distinctly recognized as such, but the nuclei of the neurilemma are often rendered plainly visible.

No. 30.—*Exhibition of the Axis-cylinder with Chromic Acid*.—Expose the sciatic nerve of a rabbit recently killed, *being careful not to touch it*; place a match-stick parallel to the long axis of the nerve, and secure it by means of ligatures at the upper and lower ends; cut the nerve on the further side of each ligature, and place it, with the wood, in 100 c.c. of a 0.1 per cent. chromic-acid solution (p. 20).

In about 24 hours cut the ligatures and tease a piece of the nerve, 0.5 to 1 cm. long, separating it into bundles, not fibers. Put the bundles back into the chromic-acid solution; in 24 hours transfer them to 50 c.c. of distilled water, and in 2 to 3 hours to 30 c.c. of gradually strengthened alcohols to harden (p. 29). It is advantageous to leave the bundles for a long time, 1 to 8 weeks, in 90 per cent. alcohol, as they are then more readily stained. After

FIG. 261.—NERVE FIBER OF RABBIT. $\times 560$.

the hardening is completed, the bundles are to be teased in a drop of picrocarmine, placed in the moist chamber, and after the staining is completed, (which, according to the length of time the tissue was allowed to harden in the alcohol, requires from $\frac{1}{2}$ to 3 days) preserved in acidulated glycerine (p. 41). The nodes of Ranvier are not as distinct as in fresh and in osmic-acid preparations, but appear as delicate transverse lines (Fig. 261). The somewhat shrunken axis-cylinder and the nuclei are stained a fine red. The intensity of the color depends on the quality of the picrocarmine, which unfortunately varies greatly.

No. 31.—*Nodes of Ranvier and Axis-cylinders*.—Add 10 c.c. of a 1 per cent. solution of silver nitrate to 20 c.c. of distilled water. Kill a frog, open the abdomen by a crucial incision, turn out the viscera, and expose the nerves descending on either side of the vertebral column. Wash out the abdominal cavity with distilled water, and pour over the nerves about one-third of the silver solution. After two minutes carefully cut out the delicate nerves, put them for a half-hour in the remainder of the silver solution, *placing them in the dark*. Then transfer them to 10 c.c. of distilled water, in which they may remain for from 1 to 24 hours. If the nerves are now examined in a drop of water, with the low power, the endothelial sheath of the nerve and numerous pigment-cells will be seen; frequently a blood-vessel lies along the nerve. On examination with the high power, little will be seen of the nodes and axis-cylinders, but if the preparation be exposed for several hours to daylight (or a few minutes to sunlight) the reaction takes place and the parts mentioned become silvered. The biconical swelling on the axis-cylinder often becomes displaced in teasing, and cannot always be readily found by the beginner (Fig. 42).

No. 32.—*Nonmedullated Nerve-fibers*.—Tease a portion of the pneumogastric nerve of a rabbit on a dry slide (No. 27 *a*), and add a few drops of a $\frac{1}{2}$ per cent. osmic-acid solution; in 5 to 10 minutes the medullated nerve-fibers become blackened (which may be ascertained by examination with the low power). Remove the osmic-acid solution and add a few drops of distilled water, which should be renewed in 5 minutes. In 5 minutes more remove the water, add a few drops of picrocarmine, apply a cover-glass, and place in the moist chamber for from 24 to 48 hours; then displace the picrocarmine with acidulated glycerine (p. 41). The tissue may be teased again after the staining is completed, which is now more easily done because the elements are more distinctly seen. With high magnification the medullated nerve-fibers appear blue-black, the nonmedullated pale gray and finely striated longitudinally. The sympathetic nerve treated in the same way exhibits more numerous non-medullated nerve-fibers. But this nerve is somewhat more difficult to find. Cut through the greater cornu of the hyoid bone, also the hypoglossal nerve, and push them aside; behind the pneumogastric nerve lies the sympathetic, which may be recognized by its 3 to 4 mm. in size, ellipsoidal, yellowish, and transparent superior cervical ganglion. If the piece of the nerve lying close under the ganglion be teased, ganglion-cells, the majority of which contain two nuclei, will be obtained; it is difficult to isolate the cells so that their processes can be seen. In Fig. 41, accidentally, only the more unusual uninucleated ganglion-cell is to be seen.

VI. THE HEART AND THE BLOOD-VESSELS.

No. 33.—*The Heart and the Large Blood-vessels*.—Cut out a papillary muscle from a human heart, a piece of the aorta 2 cm. long, a piece 1 to 2 cm. long of the bronchial artery with its veins and the surrounding connective

tissue, and a piece of the renal vein 1 cm. long, and suspend them on a thread in a bottle containing 40 c.c. of absolute alcohol. In 24 to 48 hours the objects are ready to section. Embed them in liver (the artery and vein may be embedded together and will not be injured by strong compression), cut thin cross-sections and stain in Böhmer's hematoxylin 2 to 5 minutes (p. 31). Mount in damar (Fig. 43, 45, 47, 48, 49). The elastic fibers do not stain, but with the high power can often be distinctly recognized.

The arrangement of the elements of the adventitia cannot be satisfactorily appreciated in cross-sections—often they all appear to be circularly disposed (a portion of them are circularly arranged—for example, those of the innermost strata of the external elastic membrane). The exact arrangement can only be seen in longitudinal sections, which also show the muscle-fibers of the adventitia plainly.

No. 34.—*Small Blood-vessels and Capillaries*.—From the base of a human brain strip off slowly pieces of the pia 1 to 3 cm. in length (in this way delicate blood-vessels that penetrate the brain vertically are obtained), shake them in Müller's fluid to free them from adherent fragments of brain-tissue, and place them in 50 c.c. of Müller's fluid for from 3 to 10 days; then transfer them for from 1 to 3 hours to water (for 1 hour to running water), and harden them in about 40 c.c. of gradually strengthened alcohol (p. 29). Examine one of these pieces in a watch-glass on a black background, and it will be seen that small vessels are isolated.

a. With a fine scissors cut off small twigs with their ramifications, stain them 2 to 5 minutes in Böhmer's hematoxylin (p. 31) and mount in damar (Fig. 44).

b. From the larger twigs of the cerebral blood-vessels cut pieces about 5 mm. long, slit them open lengthwise, stain them in Böhmer's hematoxylin, and place them on the slide with the adventitia side down. Mount in damar. By changing the focus the three coats of the vessels and their general arrangement can be seen.

Capillaries may also be found on examining fresh brain tissue. They may be recognized by their parallel outlines and the oval nuclei of their endothelial cells; they may be found in other preparations, for example in No. 9.

No. 35.—*Epithelium (Endothelium) of the Blood-vessels*.—Decapitate a rabbit, open the abdomen by a crucial cut made with the scissors; insert under the mesentery a cork frame about 2 cm. square, span it smoothly over this and fasten it with quills or hedge-hog spines, taking care to touch the membrane as little as possible with the fingers. Cut it off all around the frame and place the stretched membrane with the frame in 20 to 30 c.c. of 1 per cent. silver solution. In about 30 seconds the solution becomes turbid and milky; remove the frame, carefully wash the membrane with distilled water, place the whole in a white capsule containing 100 c.c. of distilled water and expose it to direct sunlight. In a few minutes a brown coloration appears. Now transfer the whole to 50 c.c. of 70 per cent. alcohol (the membrane must be submerged in the alcohol); in a half-hour cut out small pieces, 5 to 10 mm. long, and mount them in damar. In the absence of sunlight, take the preparation from the silver solution, wash it; place it for about 20 hours in about 30 c.c. of 70 per cent. of alcohol, then in a like quantity of 90 per cent. alcohol, and expose it to sunlight on the first opportunity. It must not be forgotten that the whole blood-vessel and not a section of it is present, so that in order to obtain a view such as that in Fig. 46, the surface of the vessel must be in focus.

No. 36.—*Elastic Fenestrated Membranes*.—See Techn. No. 13.

No. 37.—*Development of Capillaries*.—Chloroform a seven-days-old rabbit, fasten it with pins on a cork-plate, open the abdomen by a crucial incision, quickly remove the spleen, stomach, and attached greater omentum and place these parts in 80 c.c. of a saturated aqueous solution of picric acid (p. 20). In this solution the omentum, otherwise difficult to separate, spreads out easily. After 1 hour cut it off, transfer it to 60 c.c. of distilled water, and divide with the scissors into pieces about 1 cm. square. Place such a piece on a dry slide (remove the water with filter-paper) and with needles spread it out as smooth as possible, which is the more easily done, the less moisture there is present. Put 1 to 2 drops of Böhmer's hematoxylin on the preparation. In from 1 to 5 minutes drain off the hematoxylin and place the slide with the preparation in a flat dish containing distilled water; the membrane will soon float from the slide, but will remain smooth, and in 5 minutes should be transferred with the section-lifter to a watch-glass containing eosin (p. 23), in which it should remain 3 minutes. It should then be washed for 1 minute in distilled water and placed on a slide; the water should be absorbed with filter-paper, any wrinkles smoothed out with needles, and a cover-glass with a drop of dilute glycerine suspended from its lower surface applied. The preparation may be mounted in damar instead of glycerine (that is, dehydrated in absolute alcohol, cleared in oil of bergamot, and then mounted in damar), but the finer structural details are apt to be lost. The colored blood-corpuscles are stained a bright red by the eosin (Fig. 50).

In spreading out the membrane on the slide, delicate young capillaries may be easily torn loose from the older capillaries and then simulate "isolated cells containing blood-corpuscles;" such artificial products have been described as "vasoformative cells."

VII. THE BLOOD.

No. 38.—*Colored Blood-corpuscles of Man*.—Carefully cleanse a slide and a small cover-glass (finally with alcohol). With a clean needle prick the tip of the finger, at the side; with the cover-glass lightly touch the first drop of blood that exudes, and without the addition of any reagent place it immediately on the slide. With the high power many colored corpuscles adhering to one another by their broad surfaces, forming the so-called rouleaux, may be seen, as well as isolated colored and colorless blood-corpuscles. The distortion of many of the colored corpuscles is due to evaporation; the corpuscles are beset with minute spines, a condition which is known as crenation. If a drop of water be placed at the edge of the cover-glass, the corpuscles soon become decolorized and the water acquires a yellowish tinge; the corpuscles become spherical, have the appearance of pale circles, and finally disappear entirely. The student is advised to study the decolorization of a single corpuscle. In Fig. 51, 6, the tinged area surrounding the bleached corpuscles is somewhat too deeply shaded.

No. 39.—*Permanent preparations of colored and colorless blood-corpuscles* are made by Ehrlich's dry method. The method accurately carried out, after some practice, yields good results, but with unskilful manipulation many caricatures arise and mislead the inexperienced. The employment of this method for purposes of investigation and discovery requires great skill and great caution in judgment.

Preliminary Manipulations.—For each preparation two thin cover-glasses are required (they must not be over 0.1 mm. thick). Place them for a few

minutes in dilute hydrochloric acid, then in distilled water, and finally in alcohol. It is best to take cover-glasses that have never been used. Prepare a mixture of equal parts of absolute alcohol and ether (about 5 c.c. of each). Cleanse the tip of the finger first with soap and water, and then with a tuft of clean cotton-wool moistened in the alcohol-ether mixture. With a clean needle (not previously used for anatomic purposes) prick the pad of the finger, made slightly hyperemic by compression; take up a cover-glass with the forceps (not with the fingers) press it lightly upon the blood that exudes, and place it on the second cover-glass, with one edge projecting slightly. The drop of blood will spread out in a thin film between the two glasses, which are then *slipped* apart by means of two forceps. By this manipulation the influence of the insensible perspiration on the blood-corpuscles is prevented, which otherwise would shrink or lose their hemoglobin.

Exposed to the air, the blood on the cover-glasses dries in a few minutes; the glasses are then to be placed in the alcohol-ether mixture for fixation. In from $\frac{1}{4}$ to 2 hours they should be removed, dried again in the air, when they are ready for further treatment, which may be applied immediately or later, since the preparations thus fixed may be preserved for a long time.

a. Oxyphile (Eosinophile, α) Granules.—Place the cover-glass preparation for 24 hours in about 4 c.c. of distilled water, to which about 10 drops of eosin solution have been added. Rinse 1 minute in distilled water and stain 1 to 5 minutes in a watch-glass with hemalum (p. 32). Transfer to distilled water; remove in 5 minutes and let the preparation dry in air under a bell-glass. Mount in damar. The colored blood-corpuscles and the oxyphile granules of the colorless corpuscles are stained a bright red, the nuclei are blue. The oxyphile granules occur in the leucocytes of normal blood, of the lymph, and in the tissues, but are uncommon in normal blood. A magnification of 400 diameters is sufficient to find them.

b. Basophile Granules.—Two groups are distinguished, the γ -granules and the δ -granules. The γ -granules (*Mastzellen Granulationen*), which occur only in the leucocytes of pathologic blood, are to be stained according to the method given in No. 6. When the staining is completed, proceed as in *a*. The blue-violet granules are coarser than the—

δ -granules, which occur in the round nucleated leucocytes of normal and other blood. Stain the cover-glass preparation 5 to 10 minutes in 5 c.c. of methylene blue solution (p. 23), wash, dry, and mount in damar. These granules are minute and scarcely to be seen with the usual high-power dry lenses; an immersion lens should be used. In staining with methylene blue not infrequently the film of blood floats from the cover-glass; this may be prevented by passing the dry cover-glass preparation rapidly through a flame before staining.

c. Neutrophile (ϵ -) Granules.—Dissolve (1) 1 gm. of orange-yellow extra in 50 c.c. of distilled water; (2) 1 gm. of acid fuchsin extra in 50 c.c. of distilled water; (3) 1 gm. of crystallized methyl-green in 50 c.c. of distilled water, and let the three solutions settle. Then mix 11 c.c. of solution (1) with 10 c.c. of solution (2), and add 20 c.c. of distilled water and 10 c.c. of distilled alcohol; to this mixture add a mixture of 13 c.c. of solution (3), 10 c.c. of distilled water, and 3 c.c. of absolute alcohol. The whole should then be allowed to stand for from 1 to 2 weeks. In this "triacid-solution" the cover-glass should be placed for 15 minutes, then washed, dried, and mounted in damar. The neutrophile granules, which are found in the leucocytes with lobulated nuclei in normal and other blood, are of a violet color, and are easily seen with the usual dry high-power lenses; the oxyphile granules and the colored blood-corpuscles are of a yellow-brown or chocolate-brown color, the

nuclei a bright blue-green, though their outlines are not so distinct as in the hemalum preparation.

No. 40.—*Blood-platelets*.—Mix about 5 drops of an aqueous solution of methyl-violet (p. 23) with about 5 c.c. of salt solution (p. 19). FILTER the mixture and place a drop of it on the tip of the finger; prick the finger through the drop; the blood as it exudes mixes with the methyl-violet; take up a drop of it with the cover-glass, and examine with the high power. The platelets are stained an intense blue, are of a peculiar luster, disk-shaped, and not to be confused with the white corpuscles, likewise stained blue (Fig. 51). They are numerically variable elements, occurring in large numbers in the blood of one individual, while in the blood of another they are only to be found singly here and there. Care must be taken not to confuse them with foreign particles, which may occur even in the filtered staining solution.

No. 41.—*Colored Blood-corpuscles of the Frog*.—Prepare the slide and treat the blood like No. 38.

No. 42.—*For Legal Purposes*.—Since it is usually dried blood that is to be examined, dissolve small particles of dried blood in 35 per cent. potash solution on a slide; blood-stained pieces of linen may be teased in a drop of the same solution. Although the colored blood-corpuscles of domestic mammalian animals are smaller than those of man, it is nevertheless impossible from the size of the blood-cell to determine its source. On the other hand, it is easy to distinguish the disk-shaped corpuscles of mammals from the oval elements of other vertebrates.

No. 43.—*Colorless Blood-corpuscles, Leucocytes in Motion*.—*Preliminary manipulations*: carefully cleanse a slide and cover-glass with alcohol. Kill a frog, grasp it by its hind legs, dry its back somewhat with a cloth, and with fine scissors make an incision 1 cm. long parallel to and close beside the vertebral column. Introduce a capillary pipette into the wound (with the tip directed forward) and suck the tip full. A small drop is sufficient; blow it on to the slide, cover it quickly, and seal the edges with melted paraffin (p. 41). Such a preparation shows colored and colorless blood-cells; at first the nuclei of the former are indistinct. The nuclei of the living colorless blood-corpuscles are in general not to be seen. For the study of amoeboid movement, select leucocytes whose protoplasm is partly granular and that are not spherical. The movements are slow; of this one may convince one's self by studying a single leucocyte and making sketches of it at intervals of from 1 to 2 minutes. Study with the high power (Fig. 4).

No. 44.—*Blood Crystals*.—*a. Hemin crystals* are easily obtained. Cut a small strip, about 3 mm. long, from a piece of linen previously saturated with blood and dried, and place it with a pinhead-sized crystal of common salt on a clean slide; add a large drop of glacial acetic acid, and with a glass rod stir the linen and salt until the acid acquires a brownish tinge. This must be done rapidly, lest the acetic acid evaporate. Heat the slide over a flame until the fluid boils up once (this may be most readily seen near the strip of linen). Remove the linen and examine the dry brown places on the slide with the high power (from 240 diameters up). Occasionally the *brown crystals* may be seen without the cover-glass and without a mounting medium, lying next to numerous fragments of white salt crystals (Fig. 53, 1). To preserve, add a large drop of damar and apply a cover-glass. The hemin crystals vary greatly in form and size. In the same slide well-developed crystals lying singly, crosswise over one another, or in stellate groups may be seen, with whetstone shapes and minute par-

ticles that scarcely exhibit crystallization. The demonstration of the hemin crystals is of great importance in forensic cases. While it is easy to exhibit the crystals in large stains on wearing apparel, it is difficult when the stains are small, and especially on rusty iron, to prove that they are from blood. The instruments and reagents employed in such investigations must be absolutely free from contamination.

b. Hematoidin crystals are obtained by teasing old blood extravasations; they can be recognized macroscopically by their reddish-brown color (in the corpus luteum, in cerebral hemorrhages).

c. Hemoglobin crystals may be obtained by transferring 5 c.c. of the blood of a dog to a test-tube, adding a couple of drops of ether, and shaking vigorously until the blood becomes lake-colored. Then spread a drop on a slide and let the preparation dry in the cold. When crystallization has occurred, add a drop of glycerine and apply a cover-glass. The large crystals often exhibit a tendency to split lengthwise (Fig. 53, 4 *a*).

VIII. THE LYMPHATIC SYSTEM.

No. 45.—*Lymph-vessels*.—For the study of the *walls* of the larger lymph-vessels select the vessels opening into the inguinal glands, that are large enough to be taken out with the forceps and scalpel. Prepare like the large blood-vessels, No. 33 or No. 34 *b*.

No. 46.—For the representation of the more *delicate* lymph-vessels, their course and arrangement, the method of interstitial injection is often employed. The needle of a hypodermic syringe filled with Berlin-blue is thrust haphazard into the tissue; this is a crude method, the results of which are of very doubtful value. Even though here and there actual lymph-vessels may be thus filled, in most cases the injection-mass is simply driven forcibly into the interfascicular clefts of the connective tissue. From this the value of any decision with regard to "lymph-vessels" and "lymph-spaces" thus exhibited may be inferred.

No. 47.—*Lymph-nodes*.—For a general view the mesenteric glands of kittens are suitable. For fixation and hardening place them in 30 c.c. of absolute alcohol; in three days thin sections can be readily made, and should be taken so that they pass through the hilus, which may be easily recognized macroscopically by an external depression. Longitudinal sections passing through the poles of the node are best, though transverse sections are also useful. Stain 6 to 8 sections in Böhmer's hematoxylin for from 2 to 3 minutes, then in eosin, at the most 1 minute (p. 32, 3 *b*), then transfer them to a test-tube half filled with distilled water and shake them for from 3 to 5 minutes. Pour the shaken sections into a flat dish; the cortex and medulla can be distinguished macroscopically by the uniformly blue color of the former and the variegated appearance of the latter. Mount in damar. With the lower power fields similar to that in Fig. 55 may be seen in favorable places. The trabeculae are but slightly developed. The adipose tissue adhering to the nodes must not be taken for reticular tissue. High magnification is of no advantage; the sharp outlines disappear and the picture loses in distinctness.

No. 48.—*Lymph-nodes of mature animals and of man* are difficult to understand, because the entire cortex is transformed into a continuous mass sprinkled with irregular germinal centers. In shaking the sections the germinal centers are apt to fall out, and leave round spaces recognizable macroscopically. The lymph-sinuses can only be indistinctly made out. The mesenteric fol-

cles of the ox are well adapted for the representation of the network of the *medullary cords* and *trabeculae*. Place pieces 2 cm. long in 200 c.c. of concentrated aqueous picric-acid solution, and after 24 hours, with a sharp knife moistened with water, endeavor to cut thin sections. This is not so easily done as after alcohol fixation, but slightly thicker sections can be used. Place the sections for one hour in 100 c.c. of distilled water, which must be changed frequently, then stain with Böhmer's hematoxylin and eosin and shake them (see No. 47). Mount in damar (p. 38). The trabeculae are red, the medullary cords blue; with low magnification the appearance of the section is like Fig. 56; with high magnification the reticular connective tissue of the lymph-sinuses can be seen; the majority of the leucocytes occupying the meshes become loosened by the treatment with picric acid and lost in the shaking.

No. 49.—*Elements of the Spleen*.—Make an incision through a fresh spleen; with a scalpel obliquely applied scrape the cut surface and examine a little of the red mass adhering to the blade in a drop of salt solution. Use the high power. Often, especially in animals, only colored and colorless blood-corpuscles are found; some of the latter contain minute granules. In human spleens, in addition to the numerous colored blood-corpuscles altered in form, endothelial cells of the blood-vessels may be found; the latter were formerly called "spleen-fibers" (Fig. 58, 2, 3). In many human spleens, multinucleated cells and cells containing colored blood-corpuscles often cannot be found (Fig. 58, 4).

No. 50.—*The Spleen*.—Without cutting it, fix the entire spleen in Müller's fluid, using one liter for a human, 200 to 300 c.c. for a cat's spleen. After 2 weeks for the cat's, 5 weeks for the human spleen, wash for from 1 to 2 hours in, if possible, running water, cut out pieces 2 cm. square and harden them in 60 c.c. of gradually strengthened alcohol (p. 29). Sections not too thin are to be stained in Böhmer's hematoxylin and mounted in damar. If it is desired to stain the trabeculae, after staining in hematoxylin is completed place the sections for $\frac{1}{2}$ minute in eosin. In successful preparations the pulp and the Malpighian bodies are blue, the trabeculae rosy, the vessels, distended with blood-corpuscles, brown. If the staining in eosin be prolonged beyond 30 seconds the blood-corpuscles become brick-red, the trabeculae dark red, and the distinction between them is apt to be lost. The sections are most satisfactory when examined with a very low power (Fig. 57); with the high power the outlines are often indistinct.

No. 51.—*Reticular Connective-tissue of the Spleen*.—Shake a thin section fixed and stained like No. 50 for about 5 minutes in a test-tube half filled with distilled water. Mount in glycerine. The leucocytes are difficult to dislodge; the narrow-meshed network can only be seen at the edges of the preparation (Fig. 59).

No. 52.—*Karyomitotic Figures in the Spleen and Lymph-nodes*.—For this purpose small pieces (5 to 10 mm. long) of *warm living* spleen and lymph-node should be fixed in chromo-aceto-osmic acid (p. 21), and hardened in alcohol. Stain thin sections in safranin (p. 33). Mount in damar. The karyomitotic figures of mammals are so small that, with the usual magnification (560 diameters), they can only be found by the practiced microscopist. They may be recognized by their deep red color (Fig. 60).

No. 53.—*Blood-vessels of the Spleen* may be obtained, incidentally, by injecting the stomach and intestine (compare with No. 110).

No. 54.—*Nerves of Spleen*.—For this purpose the spleen of the mouse is best suited. Halve it, and apply Golgi's method for demonstration of the ele-

ments of the nervous system (p. 35). It is sometimes sufficient to place the object in the osmio-bichromate mixture (in a warm chamber) for 3 days and for the same length of time in the silver solution; often a repetition of the whole process once or twice yields good results.

IX. BONE.

No. 55.—*Dried Bone*.—The bone must not be dried before maceration, but must be placed fresh for several months in water, which should be frequently changed. Then it is to be dried and a piece held between two pieces of cork or cloth clamped in a vice, and with a compass-saw sections 1 to 2 mm. thick, transverse or longitudinal, are to be cut. Secure a section with sealing-wax to the under surface of a cork-stopper, dip the whole for a moment in water and then file it, first with a coarse, then with a fine file, until it is perfectly smooth: the file must be dipped in water frequently, in order to wash off the adherent particles of bone and to prevent the heating of the sealing-wax by friction.

The section of bone should then be loosened by heating the sealing-wax, and the smooth side stuck fast to the stopper. It must now be filed until it is so thin that the sealing-wax can be seen through it. The whole should then be placed in 90 per cent. alcohol, in which within a few minutes the section becomes loosened from the cork. Moisten a coarse whetstone with water, rub it with a second whetstone until the surface is covered with a little grinding-paste; lay the section in it, place a smooth cork upon it (one without cracks), and with a circular motion grind it on both sides; it is not necessary to glue the section to the cork. The section when sufficiently thin is transparent; this is to be ascertained by drying it between pieces of filter-paper and examining with the low power. It should then be ground on a fine whetstone, in the same manner as on the coarse, and when both sides are smooth, dried with filter-paper and polished. To do the latter, nail a piece of wash-leather smoothly on a board, sprinkle it with chalk, and with the tip of the finger rub the section to and fro on it. In this way the previously dull section acquires shining surfaces. The adherent powder may be removed by rubbing the section on fresh wash-leather. The finished section is to be placed dry on a slide and the cover-glass secured by means of cement (p. 38).

Examine first with the low, then with the high power. (If the section is thick, it may be impossible to examine it with the high power, since then the objective cannot be brought near enough to the preparation.) The bone lacunæ and bone canaliculi are filled with air, and with the customary illumination of the object from below appear black (Fig. 28).

No. 56.—*Sharpey's Fibers*.—Prepare a cross-section of the middle of the shaft of a tubular bone, preferably of a young individual, according to the method given in No. 55. Place the finished dry section for from 2 to 5 minutes in 4 c.c. of turpentine and then mount in damar. The fibers, invisible in the sections produced by other methods (No. 55 and 57), can be plainly seen, even with the low power (Fig. 66).

No. 57.—*Haversian Canals and Lamellæ*.—Select the metacarpal bone of an adult: after 4 weeks' fixation in Müller's fluid, and hardening in alcohol, decalcify in nitric acid (p. 29), harden again, and cut transverse and longitudinal sections. The compact structure of larger bones (the femur, for example) require too much time (several weeks) for decalcification. The periosteum should be allowed to remain on the bone. For longitudinal views of Haversian canals very thick sections (0.5 mm. and more) must be cut.

Mount in dilute glycerine (Fig. 63). Neither are very thin sections necessary for transverse views and lamellar systems; the lamellæ are best seen if the section be examined in a drop of distilled water and the mirror turned so that the object is only half illuminated; thus, too, the striæ produced by the bone canaliculi, running vertical to the lamellæ, are best seen (Fig. 64). Mount in dilute glycerine; this, however, renders the lamellar systems partially indistinct. Not every part of the bone exhibits all the lamellar systems; the outer and also the inner ground lamellæ are frequently wanting. In sections taken near the epiphyses the continuation of the compact substance into the trabeculæ of the spongy bone may be seen. The bone lacunæ and bone canaliculi are much less distinct in moist preparations than in dried ground sections, because the contained air has been displaced by the mounting medium. (Compare Fig. 28 and 29.)

Not infrequently the concentric lamellæ of the Haversian systems are found to be interrupted by an irregular line. Up to this line the osseous tissue previously formed has been again resorbed. All that which lies within the line is newly-deposited bone-substance. These formations are, therefore, partially filled Haversian spaces (Fig. 64, *h*).

FIG. 262.—ISOLATED ELEMENTS OF FRESH BONE-MARROW FROM A VERTEBRA OF CALF. $\times 560$. 1. In salt solution. 2. Stained with picrocarmine. 3. After treatment with acidulated glycerine. *k*, Marrow-cells; *k'*, two marrow-cells containing masses of pigment-granules, the cell on the right seen from the side, the cell on the left, from the surface; *b*, nonnucleated colored blood-corpuscles; *r*, giant-cells; in the one on the right the nucleus is dividing by constriction, and two of the future new nuclei are seen from the side, another, *x*, from the surface.

No. 58.—*Red Bone-marrow*.—*a*. Compress the vertebra (cut in half) or the rib of a calf in a vice or with tongs; with a pipette take up a small drop of the liquid thus expressed, transfer it to a slide and, without the addition of any other fluid, apply a small cover-glass, or better, a fragment of a cover-glass. Examined with the high power red blood-corpuscles, hematoblasts, marrow-cells of different sizes, and giant-cells will be seen, but not always their nuclei (Fig. 262, 1). Add a drop of picrocarmine (p. 41); the nuclei become red in from 1 to 2 minutes, but are still pale (Fig. 262, 2). If the picrocarmine is displaced by salt solution and then by dilute acidulated glycerine, the nuclei acquire a deep color and sharp contours (Fig. 262, 3). Occasionally giant-cells are sought in vain. Human ribs are often usable.

b. To make permanent preparations, proceed as follows: With a thin cover-glass take up a drop of the marrow expressed from a rib and make two cover-glass preparations as directed in No. 39. Since the marrow does not diffuse as readily as the blood between the two cover-glasses, make slight pressure upon them before slipping them apart. They should not be allowed to dry, but should be placed at once in a concentrated aqueous solution of sublimate solu-

tion (5 gm. in 100 c.c. of distilled water). At the end of 10 minutes transfer the cover-glasses to 20 c.c. of distilled water, which is to be changed in about 5 minutes. In 10 minutes place them in 5 c.c. of diluted eosin (p. 32, 3 b) for from 1 to 5 minutes, then wash for a moment in distilled water and transfer them to 5 c.c. of filtered Böhmer's hematoxylin; after 1 to 2 minutes place them for 5 minutes in distilled water; remove the water by means of filter-paper placed at the edge of the cover-glass, and place them in absolute alcohol (not longer than 1 minute, lest the eosin be extracted), then in pure oil of bergamot for 3 minutes. With a cloth carefully remove the oil from the film-free surface of the cover-glass, place a drop of damar on the surface containing the film of marrow, and invert the cover-glass on a slide. The colored blood-corpuscles and the protoplasm of the hematoblasts are stained a brilliant red, the protoplasm of the remaining cells gray-violet; all the nuclei are blue. Cells containing oxyphile (eosinophile) granules are often found (Fig. 65). The colored blood-corpuscles frequently exhibit distorted forms.

No. 59.—*Articular Cartilage*.—Select the head of the metacarpal bone of an adult, and treat it according to the method given in No. 57. Cut longitudinal sections and mount them in dilute glycerine (Fig. 67). The parallel streaks often present in the hyaline cartilage are produced by the razor. The granules of the calcified cartilage have disappeared in consequence of the process of decalcification to which the tissue was subjected.

No. 60.—*Synovial Villi*.—From a cadaver, as fresh as possible, cut out a piece about 4 cm. long of the capsular ligament at the edge of the patella, and with the scissors cut a strip 2 to 3 mm. broad from the reddish, glossy, velvety inner surface of the same, moisten it with a drop of salt solution, and without a cover-glass examine it with the low power. At the edges of the tissue the villi may be seen; their blood-vessels often still contain blood-corpuscles. The refractive nuclei of the endothelial cells lie close beside one another (Fig. 68).

If it is desired, the preparation may be stained under the cover-glass with picrocarmine and mounted in diluted glycerine (p. 41), but much of the original beauty is thus lost.

No. 61.—*Development of Bone*.—Human embryos of 4 to 5 months, embryos of the sheep, pig, or cow, 10 to 14 cm. long (measured from the tip of the snout to the root of the tail), are suitable. The latter may be obtained at the slaughter-house; the entire uterus should be ordered. Place the embryos in toto (2 to 3 in 1 liter) in Müller's fluid for 4 weeks; the fluid must be changed frequently. Then wash in running water 1 to 6 hours, and harden in 200 to 400 c.c. of gradually strengthened alcohol (p. 29). After the embryos have lain 1 week or longer in 90 per cent. alcohol, cut off the head and the extremities, close to the rump, and decalcify them in 200 c.c. of distilled water, to which 2 to 4 c.c. of pure nitric acid have been added. In 2 to 5 days, during which the decalcification medium must be changed about three times, the extremities are to be taken out (the head is probably not yet decalcified, and must remain in 2 per cent. nitric acid another several days) and washed 1 to 6 hours in running water, and again hardened in gradually strengthened alcohol. After they have lain 5 days in 90 per cent. alcohol, cut the extremities into pieces 1 cm. long, which, should they still be too soft, may be placed for 1 to 2 days in 30 c.c. of absolute alcohol.

The vertebrae and the ribs furnish instructive specimens.

To obtain sections showing the *first processes* in the development of bone, embed in liver the phalanges and metacarpal bones (the latter are very long in

the animals mentioned), and make longitudinal (sagittal) sections, from the flexor to the extensor surface; to be good sections must be taken in the axis of the extremities; those taken from the margin exhibit pictures that are not intelligible.

For *more advanced stages* make chiefly transverse sections of the humerus and femur. Sections through the diaphysis show more perichondral, sections through the epiphyses more endochondral bone.

The most beautiful examples of *osteoblasts* may be obtained in cross-sections of the inferior maxilla; they are also valuable as preparations showing the development of teeth.

For still *later stages* the skeleton of newborn animals is useful; their phalanges show tolerably early stages in the process, their carpal bones the first stages. The decalcification requires somewhat more time (up to 8 days).

For *intermembranous bone* select the parietal and frontal bones of embryos; make horizontal sections.

The sections are to be stained in 4 c.c. of Böhmer's hematoxylin, 2 to 10 minutes, transferred to 10 c.c. of distilled water for 10 minutes, then to 4 c.c. of picrocarmine for 10 minutes (pp. 31, 33), to 20 c.c. of distilled water for from 15 minutes to 1 hour, and mounted in damar (p. 38).

If the staining is successful, the cartilage (especially the calcified portions) is blue, the bone red. Occasionally the cartilage does not stain well; then place the sections in 5 c.c. of distilled water plus 5 drops of filtered hematoxylin solution. In 6 to 14 hours the cartilage will become blue. The picrocarmine staining of bone is often not uniform, the youngest portions of the bone, the margins of the osseous trabeculae, for example, are often the more brilliantly stained.

X. MUSCLES AND TENDON.

No. 62.—*Bundles of Striped Muscle*.—Select a muscle in which the fibers have a parallel disposition (for example, the adductor of the rabbit) and with a sharp razor make a deep incision transverse to the course of the fibers and 2 to 3 cm. below a second incision; connect these by longitudinal incisions and, without traction, carefully remove the area thus mapped out. For fixation place it in 100 c.c. of 0.1 per cent. chromic acid (p. 27). After 2 weeks wash it in running water and harden in 50 c.c. of gradually strengthened alcohol (p. 29). Make cross-sections and examine in diluted glycerine (Fig. 76). The muscle-fibers vary greatly in thickness; the smallest are sections through the ends of the fibers. Although the muscle-fibers are cylindrical and should therefore in section appear circular, they have an irregularly polygonal outline due to mutual pressure. The color of the sections is very different—some are quite dark, others quite clear. The cause of this phenomenon is unknown to me. The endomysium is best seen with the high power (240 diameters).

No. 63.—*Tendons*.—Cut from a tendon a piece 5 to 10 cm. long, and let it dry in the air (but not in the sun). Thin tendons at room-temperature are sufficiently dry in 24 hours. Thicker tendons require several days. With the scalpel (not the razor) cut a smooth transverse surface and then cut thin shavings from the tendon, supporting it on the thumb of the right hand and with the remaining fingers grasping the scalpel (the manipulation is the same as in sharpening a pencil). Throw the majority of the shavings into a capsule containing distilled water, and in 2 minutes examine in a drop of the same medium (Fig. 77, A). To preserve, stain in 3 c.c. of picrocarmine for 5 minutes and mount in dilute glycerin. Very frequently a streak may be seen extending

across the entire section; this has been produced by the knife. Place a second section, unstained, in a drop of water on a slide; treat it under the cover-glass with a drop of acetic acid; the edge of the section soon exhibits swollen convoluted bands (acetic-acid reaction of connective tissue).

No. 64.—For the study of the *minute structure of tendon, its cells and their processes*, place a thin tendon, as fresh as possible (that of the palmaris longus muscle) in pieces 3 cm. long in 100 c.c. of 0.5 per cent. chromic acid for at least 4 weeks; the chromic acid should be changed several times during this period. Then wash the tissue in running water, 1 to 2 hours, and harden it in about 40 c.c. of gradually strengthened alcohol (p. 29). The sections should be cut with a very sharp razor; often the tendon is so brittle that it falls to pieces in cutting. The sections need not be very thin. Mount them unstained in diluted glycerine. Examined with the low power and reflected light (with the mirror muffled) they yield beautiful pictures, better than the preparations made like Techn. No. 63. With the high power they resemble Fig. 77, B. The black zigzag spaces are in part occupied by tendon-cells.

No. 65.—*Tendon-cells*.—From the tail of a rat or mouse cut pieces of tendon 0.5 to 1 cm. long, and place them in 5 c.c. of alum-carmine. The following day (or later) transfer the swollen pieces to a dry slide and rapidly tease them (p. 25). It is not necessary to separate the tendon into very small bundles, but care should be taken that the bundles lie straight. Then cover the preparation with a drop of distilled water and a cover-glass. With the low power the rows of cells may be seen, appearing for the most part as dark streaks; these are the cell-nuclei seen in profile. In surface views the nuclei appear dull red. The body of the cells, the protoplasm, can only be seen with the high power; viewed laterally, it appears as a sharp, dark streak, from the surface, pale and delicate. Not infrequently the cells are folded, so that they are visible partly from the edge and partly from the surface. The connective-tissue fibers may be distinguished occasionally as delicate parallel lines; the fine elastic fibers with their sharp contours are always distinct. The focus should be changed by means of the micrometer-screw, and all the different planes of the section examined. If the cells are not distinct add a drop of acetic acid (p. 41). To preserve, displace the water with diluted glycerine.

No. 66.—*Muscle and Tendon*.—Remove the skin from the hind leg of a frog just killed, and with scissors cut off the leg above the knee-joint—that is, above the origin of the gastrocnemius. Fix it in 50 c.c. of Kleinenberg's picrosulphuric acid (p. 28). After 24 hours transfer it directly to 50 c.c. of 70 per cent. alcohol for gradual hardening. In about 6 days cut off the muscle with a piece of the tendo-Achillis, and stain it in bulk in borax-carmine (p. 32). Then harden again in 90 per cent. alcohol. Cut sagittal longitudinal sections, placing the razor on the tendon on the posterior surface of the muscle. Mount in damar (p. 38). Very often not a trace of the cross-striation of the muscle-fibers is to be seen.

XI. THE ORGANS OF THE NERVOUS SYSTEM.

No. 67.—*The Spinal Cord*.—For the study of the distribution of the white and gray substance the spinal cord of a child should be fixed in toto in about 1 liter of Müller's fluid, which should be frequently changed; after 4 or 5 months thick cross sections of the cervical, thoracic, and lumbar regions may be cut, and without further treatment mounted in dilute glycerine (p. 38), or after the customary preliminary treatment they may be mounted in damar.

No. 68.—*The Spinal Cord ; Staining of Medullated Fibers.*—The success of the preparation depends especially on the state of preservation of the organ. The fresher the tissue when it is put into the fixing fluid, the better will be the result. The entire spinal cord should be placed in a large quantity of Müller's fluid, which must be changed daily during the first week and frequently thereafter. If it is desired to investigate only portions of the spinal cord then place pieces of the fresh cord about 2 cm. long taken from the lower cervical, the middle thoracic, and the lumbar region in 200 to 500 c.c. of Müller's fluid, or better, suspend them in it. In 4 to 6 weeks, during which time the fluid must be frequently changed, the tissue is to be transferred directly, without previous washing, to 150 c.c. of 70 per cent. alcohol, and on the following day to the same quantity of 90 per cent. alcohol. The bottle containing the tissue must be placed in the dark (p. 29), and the alcohol frequently changed during the first 8 days. Sections may then be cut. The sections are to be placed in a capsule containing 20 c.c. of 70 per cent. alcohol, and as soon as possible transferred from this to 30 c.c. of Weigert's hematoxylin to which 1 c.c. of lithium carbonate solution has been added (p. 22). In 5 to 6 hours the now very dark, untransparent sections should be transferred to 50 c.c. of distilled water plus 1 c.c. of lithium carbonate solution. In a half hour, during which time the fluid must be changed several times, the sections will give off no more color and are then to be placed in 30 c.c. of potassium permanganate solution for differentiation. In $\frac{1}{2}$ to 3 minutes the sections are to be washed for 1 minute in distilled water and then transferred to 20 c.c. of the acid mixture. The capsule containing the acid mixture should be covered. The decolorization occurs in 10 to 50 seconds; the gray substance becomes light yellow, almost white, the white substance (the medullated nerve-fibers) very dark. Now transfer the sections to a capsule containing 30 c.c. of distilled water and in 5 minutes to a second capsule containing the same quantity of fresh distilled water. After 10 minutes place them in 10 c.c. of alum-carmin, in which they may remain from 3 to 15 hours. Mount in damar. The alum-carmin staining may be omitted. The foregoing directions are intended for thin well-fixed preparations. If the sections are thick, if the tissue has lain a long time in alcohol, more time will be required for staining and reduction. Should the sections not stain, place them in Müller's fluid for 24 hours, wash 1 minute in distilled water, then stain, and the result may be successful. Should the decolorization not be sufficient, if the gray substance does not become yellowish-white, the procedure may be repeated; that is, the sections are to be again placed in distilled water 1 minute, then in potassium permanganate 1 to 3 minutes, then in distilled water 1 minute, and finally in the acid mixture. The given quantities of the potassium solution, also of the acid mixture, are sufficient for only a few, about 20, sections. If it is desired to treat more sections, larger quantities of these fluids must be used.

No. 69.—*The Spinal Cord ; Staining of Axis-cylinders and Cells.*—Place pieces at the most 2 cm. long in 200 c.c. of Müller's fluid, which must be changed daily during the first week, and frequently thereafter. In 4 weeks transfer the tissue directly from the Müller's fluid to about 50 c.c. of sodium carminate (1 per cent. aqueous solution), in which it should remain for 3 days. During this time the bottle must be frequently shaken. The stained pieces are to be washed for 24 hours in running water, then placed in 150 c.c. of 70 per cent. alcohol, and after 5 hours transferred to the same quantity of 90 per cent. alcohol. Mount in damar (Fig. 86).

No. 70.—*Spinal Cord ; Golgi Staining.*—The length of time the tissue

must remain in the Golgi mixture depends upon the elements it is desired to stain, as follows:—

2 to 3 days for neuroglia cells.

3 to 5 days for nerve-cells.

5 to 7 days for nerve-fibers (collateral fibrils).

For this purpose take the spinal cord with the vertebral column of a newborn rat or mouse, and treat it according to the method given on page 35. Since the pieces must be used as soon as they are taken out of the silver solution, only one piece at a time should be transferred to the absolute alcohol. Cut the sections through the cord and the vertebral column.

The spinal cord of a 3- to 7-days'-old embryo chick furnishes still better results, but it is necessary to embed the tissue in celloidin (see Microtome Technique). The spinal cord of kittens yields good results.

No. 71.—*The Brain; Staining of Medullated Nerve-fibers.*—Apply the method given in No. 68. If an entire human brain is to be placed in Müller's fluid, many deep incisions should be made in it and about 3 liters of the fixing fluid should be used.

No. 72.—*The Brain; Cells.*—Treat pieces 1 to 2 cm. square of the cerebral cortex (paracentral convolution) and of the cerebellar cortex like No. 69. In the cerebral cortex, in addition to the cell-forms described, a variable number of vesicular spaces, containing remnants of cells (protoplasm and nuclei), may be seen. These are probably pericellular lymph-spaces, which, by post-mortem alteration and the influence of the fixation medium, have become abnormally enlarged. The sections through the cerebellar cortex must be made transverse to the long axis of the convolution, since the ramifications of the cells of Purkinje extend only in planes transverse to the convolution. In the depressions between the convolutions only a few cells of Purkinje are to be seen.

FIG. 263.—PORTION OF A SECTION OF HUMAN CEREBRAL CORTIX. $\times 240$.
p. Small pyramidal cells; a, the nerve-process of a pyramidal cell.

No. 73.—*The Brain; Golgi Staining.*—

a. For a general view, treat the brain of a newborn rat or mouse in the unopened cranium according to the method given in No. 70. The cranium may be sectioned with the brain-substance.

b. For specimens of the cortex, treat pieces of the brain of an 8- to 30-days'-old mouse with the Golgi mixture for from 2 to 3 days, or of a 1- to 15-days'-old rabbit, or a kitten under 6 weeks old, for 5 days. Pieces of the brain of adults must remain in the Golgi mixture 8 to 15 days. Further treatment like No. 70.

No. 74.—*The Cortex of the Cerebellum; Golgi Staining.*—Remove the cerebellum from the cranium of a newborn guinea pig (or a kitten less than 6 weeks old) and treat it according to the method given in No. 70. The staining of the elements of the cerebellum is more difficult to accomplish than of the cerebrum and the spinal cord. Failures are frequent. The sections should be principally made vertically to the axis of the convolutions. (For embedding, see Microtome Technique.)

No. 75.—*Hypophysis Cerebri.*—Treat like No. 80.

No. 76.—*Brain sand, Acerculus Cerebri.*—Tease the epiphysis in a drop

of salt solution. If much brain-sand is present, a gritty sound will be heard on teasing and the larger concretions can be perceived by the unaided eye. Examine with the low power, without a cover-glass (Fig. 99). Often the irregularity of the surface is indistinct. Push aside the larger granules with a needle, cover a few of the smaller ones with a cover-glass and treat with 2 to 3 drops of hydrochloric acid (p. 20). Bubbles of gas develop and the sharp outlines of the granules disappear.

No. 77.—*Corpora Amylacea*.—Select the brains of elderly individuals. With a scalpel scrape the mesial surface of the optic thalamus—that directed toward the third ventricle—and spread the scrapings with a needle in a drop of salt solution; apply a cover-glass. The corpuscles when present are easily found, and are recognized by their bluish-green color and their stratification (Fig. 100, *a*). They should not be confused with drops of extruded myelin (*b*), which are always clear and have a double contour. In addition there may be found in such preparations numerous red blood-corpuscles, ependymal cells (*d*), medullated nerve-fibers varying in thickness, and ganglion-cells; the latter are very pale and often can only be detected by their pigmentation (*f*). Human brains, even though not absolutely fresh, can still be used.

FIG. 264.—FROM A TRANSVERSE SECTION OF A PERIPHERAL (SPINAL) NERVE OF RABBIT. $\times 50$. In the lower funiculus, on the right, some of the transverse sections of nerve-fibers have fallen out, others are lying on one side, as a consequence of pressure. The endoneurium is but slightly developed in the rabbit.

No. 78.—Spread out a piece 1 cm. long of the *choroid plexus* in a drop of salt solution and apply a cover-glass. The convoluted red blood-vessels and the epithelium of the plexus can be seen.

No. 79.—*Transverse Sections of Nerve-fiber Bundles*.—Treat a piece of nerve, if possible the sciatic of man, which possesses a well-developed endoneurium, according to the method given in No. 30. Place it for 6 days in a 0.1 per cent. solution of chromic acid, then wash it for from 3 to 4 hours in running water, and harden it in gradually strengthened alcohol. When the hardening is completed, cut thin sections with a sharp razor. It is advisable to embed the tissue in liver; better still, in elder pith, or in the pith of the sunflower. For this purpose, make a hole in the dry elder pith with a needle, and then carefully insert the nerve. Place the whole for about a half-hour in water; the pith swells and firmly grasps the nerve. Stain the sections in picrocarmine, and mount in glycerine. The length of time required for staining varies greatly. The sections must be very carefully handled and pressure with the

cover-glass must be scrupulously avoided, lest the sections of the fibers, which are not disks but short cylinders, be turned on their sides, and not a fiber in section be seen. If successful, the section will show a somewhat shrunken axis-cylinder, resembling a red nucleus, surrounded by the yellow medulla, enclosed by the reddish neurilemma. The cross-section of the nerve-fiber has been compared to a picture of the sun (*Sonnenbildchenfigur*) (Fig. 102).

No. 80.—*Spinal Ganglia*.—These are difficult to obtain. Therefore remove the Gasserian ganglion from the depression in which it is lodged (on the anterior surface of the petrous portion of the temporal bone), and place it in about 100 c.c. of Müller's fluid for fixation. After 4 weeks wash it for 3 hours in running water, and harden it in 50 c.c. of gradually strengthened alcohol (p. 29). Cut the thinnest possible transverse and longitudinal sections; stain them 30 seconds in hematoxylin, and then 2 to 5 minutes in eosin (p. 32, 3 b), and mount in damar. The ganglion-cells are pale red; the axis-cylinder deep red; the medullary sheath brownish; the nuclei blue (Fig. 103). If the section is not sufficiently thin, the large number of deeply-stained nuclei will render it difficult to see the other structures. For this reason it is better to stain thick sections in picrocarmine, 2 to 3 days, and mount them in damar. The nuclei are then not so intensely stained. Occasionally the protoplasm of the ganglion-cell contracts, and thus acquires a stellate outline (Fig. 103 x), which the beginner may easily confuse with a multipolar ganglion-cell. Fixation in Kleinenberg's picrosulphuric acid gives very good results.

T-shaped branches may be seen in preparations of the spinal cord treated as in No. 70. In young embryo chicks the spinal ganglion-cells are still bipolar. Unipolar cells are found in embryo chicks about 17 days old; transition forms between the 9th and 14th days, and in embryo rabbits 5 to 12 cm. long.

No. 81.—*Sympathetic Ganglia*.—Fix and harden the large superior cervical ganglion of the sympathetic nerve like No. 80. Here, too, on account of the abundance of nuclei, nuclear staining is applicable only to *very* thin sections. Treated according to the method given in No. 80, the processes of the multipolar ganglion-cells are not rendered distinct. For this purpose place the thinnest possible sections for 24 hours in 5 c.c. of nigrosin solution (prepared like the methyl-violet solution, p. 23); then transfer them to 5 c.c. of absolute alcohol for 5 minutes, and preserve in damar. The characteristic bundles of nonmedullated nerve-fibers, cut obliquely and transversely, can be recognized with the low power; also the ganglion-cells; but to see their processes high magnification and careful scrutiny are necessary. In many sections the processes of the ganglion-cells cannot be seen; the latter may be best exhibited according to the method given in No. 70, and a suitable object is the cervical portion of a 10- to 15-days'-old embryo chick.

No. 82.—*Simple Tactile-cells; Intra-epithelial Nerve-fibers; Cells of Langerhans; Tactile Corpuscles*.—Prepare a mixture of gold chloride and formic acid (p. 37), boil it and let it cool; then cut from the volar side of a freshly-amputated finger or toe (with scissors applied flatwise) several small pieces of the epidermis and uppermost layers of the corium, about 5 mm. long and 1 mm. broad. Carefully remove any fat attached to the under surface of the corium and place the pieces in the gold and formic acid mixture for 1 hour, *in the dark*. Then, with glass rods, transfer the pieces to 10 c.c. of distilled water and in a few minutes to fresh distilled water to which formic acid has been added, and expose the whole to daylight (sunlight is unnecessary). In 24 to 48 hours the tissue becomes dark violet. It

is now to be hardened in 30 c.c. of gradually strengthened alcohol. In 8 days the pieces may be embedded in liver and sectioned; mount in damar. The epidermis is red-violet in different tints; the nuclei are only to be seen in places and often are not perceptible; the corium is white; the capillaries, the excretory ducts of the coil-glands, and the nerves are dark violet to black. For tactile-cells the thinnest possible sections are necessary. They may often be found near the excretory ducts of the coil-glands. Care must be taken not to confuse them with shrunken epithelial cells (Fig. 106).

The *intra-epithelial nerve-fibers* appear as delicate filaments; their connection with the nerve-fibers in the corium is difficult to trace. Processes of the cells of Langerhans, in thin sections, are apt to be confused with the intra-epithelial nerve-fibers (Fig. 105).

The *cells of Langerhans* and the *tactile corpuscles* may be easily seen; in thick sections the tactile corpuscles are black (Fig. 105), in thin sections red-violet (Fig. 110).

No. 83.—*Compound Tactile-cells*.—Cut the yellowish wax-like skin, or cere, from the lateral edges of the upper beak of a duck or goose and treat pieces 1 to 2 mm. thick and 1 cm. long with 3 c.c. of 2 per cent. osmic-acid solution plus 3 c.c. of distilled water; place the whole in the dark 18 to 24 hours; then wash the pieces for 1 hour in running water and transfer them to 20 c.c. of 90 per cent. alcohol. In 6 hours the objects may be sectioned. Embed them in liver and make the sections from the corium toward the epithelium, not the reverse. The sections may be mounted unstained in damar. The olive-green tactile-cells may be readily seen, but the entrance of the nerve-fiber is difficult to find (Fig. 107). In addition, Herbst's corpuscles occur in the sections. If it is desired to stain the sections, use a nuclear staining solution (p. 31).

No. 84.—*Cylindrical End-bulbs*.—With scissors and forceps cut out pieces 1 cm. square of the scleral conjunctiva near the corneal margin of the fresh eye of a calf, taking care not to roll them. It is better to let them lie smooth on the sclera. Carefully slip the pieces, epithelial side up, from the sclera on to a cork-plate, and span them out with needles. Moisten the surface with a few drops of the vitreous humor obtained from the eye; with scissors and forceps dissect off a thin layer consisting of connective tissue and the epithelium resting upon it. This operation must be done with great care; folding and torsion of the membrane must as far as possible be avoided. The pieces, with the epithelial side up, should now be slipped on to a dry slide and spread out flat. At first they draw together, but in a moment or two the edges dry somewhat and adhere to the glass, and they can then be extended without much difficulty. The slide with the preparation is next to be placed in a glass jar containing 65 c.c. of distilled water to which 2 c.c. of acetic acid have been added. In about an hour (or later), during which time the pieces swell considerably and float from the slide, with a clean needle endeavor to remove the epithelium; it may be loosened without much trouble and floats off in fine white shreds. If this is not done cautiously the end-bulbs lying close beneath the epithelium may be torn off with it. After the pieces have lain 4 to 5 hours in dilute acetic acid transfer them with a few drops of the same fluid to a slide, apply a cover-glass and make slight pressure upon it with the outspread branches of the forceps. On examination with the low power the blood-vessels may be distinctly seen—they may be recognized by their prominent nuclei—and also the medullated nerve-fibers. Trace such a fiber until the medulla ceases; examine such places with the high power, for there the end-bulbs are most apt to be found. In most

cases nothing will be seen but the numerous nuclei, and even when a favorable situation is found the end-bulbs are so pale that it is very difficult to perceive them; the axis-cylinder, too, is often very difficult to see. Only the practised microscopist will have much success in finding them. Beginners are advised not to attempt this preparation.

No. 85.—*Corpuscles of Vater*.—These are best obtained from the mesentery of a cat, where they may be seen with the unaided eye. They appear as milky, glass-like, transparent oval spots between the strands of adipose tissue of the mesentery. Their number varies greatly. Occasionally they are very scarce and of such small size that to find them requires close searching. Cut out the portion of the mesentery containing the corpuscles, and spread them out in a drop of salt solution on a slide lying on a black background. Endeavor to remove the attached clusters of fat-cells, taking care not to prick the corpuscles. Ascertain with a low power, without a cover-glass, whether the corpuscles have been sufficiently isolated. Cover them with another drop of salt solution and a cover-glass. Pressure must be carefully avoided. The corpuscle represented in Fig. 109 was of very small size. With the high power one can distinctly see the nuclei of the cells lining the capsules; on the other hand, the oval nuclei of the inner bulb are often indistinct and pale. If it is desired to preserve the preparation, treat it under the cover-glass with 1 to 2 drops of 1 per cent. osmic acid and, after the medulla is blackened and the inner-bulb has become brown, displace the acid with very dilute glycerine. Methylene-blue staining (p. 34) is recommended.

No. 86.—*Motor Nerve-endings—a. Terminal Ramifications*.—Prepare a mixture of 24 c.c. of 1 per cent. gold chloride solution plus 6 c.c. of formic acid, boil it and let it cool; cut out small pieces, 3 to 4 cm. long, of the intercostal muscles of a rabbit, and treat them like No. 82; after the dark-violet pieces have lain 3 to 6 days in 70 per cent. alcohol, tease a muscle-bundle about 5 mm. broad in a drop of dilute glycerine to which a very small drop of formic acid has been added. It is of advantage to make slight pressure on the cover-glass. To find the terminal ramifications, trace with the low power the easily recognized black nerve-fibers (Fig. 111). The addition of another drop of acetic or formic acid often renders the elements more distinct.

b. Nuclei of the Motor-plates.—Place the anterior halves of the eye-muscles of a recently-killed rabbit in 97 c.c. of distilled water plus 3 c.c. of acetic acid. After 6 hours transfer the muscles to distilled water; with the scissors cut a thin flat piece and spread it out on a slide; the ramifications of the whitish nerves can be plainly seen with the unaided eye. With low magnification (50 diameters), the anastomoses of the nerve-bundles, as well as the blood-vessels (easily recognized by the transversely-placed nuclei of their smooth-muscle-fibers) can be seen. On account of the large number of sharply-contoured nuclei belonging to the muscles and the intramuscular connective tissue, the end-plates are not easy to find. If a nerve-fiber be traced it will soon be seen that the double-contoured medullary-sheath ceases abruptly and loses itself in a group of nuclei; these are the nuclei of the motor-plate, whose other details are not distinctly visible. The cross-striation of the muscle-fibers, which are very pale, is often indistinct (Fig. 112).

No. 87.—*The Suprarenal Bodies; General View*.—Fix the entire suprarenal body of a child in 200 c.c. of 0.1 per cent. chromic acid, and after 8 days harden it in 150 c.c. of gradually strengthened alcohol; mount unstained sections in dilute glycerine (Fig. 113 A).

No. 88.—*Elements of the Suprarenal Body.*—Tease portions of the fresh organ in a drop of salt solution. The elements are very delicate and injured cells are therefore of frequent occurrence.

No. 89.—*For the study of the minute structure of the suprarenal bodies,* place 2 cm. cubes of the fresh organs in 100 c.c. of Kleinenberg's picrosulphuric acid, and after 12 to 24 hours in an equal quantity of gradually strengthened alcohol: cut fine sections, stain them in Böhmer's hematoxylin and mount in damar (Fig. 113 B).

XII. THE DIGESTIVE TRACT.

No. 90.—*Isolated Squamous Cells from the Oral Cavity.*—With a scalpel gently scrape the upper surface of the tongue and mix the scrapings with a drop of salt solution on a slide; apply a cover-glass; in addition to isolated, pale, squamous epithelial cells, leucocytes ("salivary corpuscles") may be found: also, with more vigorous scraping, the torn apices of filiform papillæ, which not infrequently are surrounded by finely granular, dark masses of micrococci to which tufts of *leptothrix buccalis* are attached. The preparation may be stained under the cover-glass with picrocarmine and then treated with dilute acidulated glycerine, provided too many air-bubbles do not make the preservation of the preparation impossible (Fig. 6, 1).

No. 91.—*Mucous Glands of the Lips.*—These are millet-sized nodules macroscopically perceptible to touch and sight. For microscopic preparations cut from the mucous membrane of a human lower lip (not the margin of the lip) 1 cm. cubes; fix them in 50 c.c. of Kleinenberg's picrosulphuric acid and in 24 hours harden in 50 c.c. of gradually strengthened alcohol. In 3 days the tissue may be sectioned. Cut many sections, not too thin, and stain them with Böhmer's hematoxylin; place the sections in water, and with the naked eye select those which include the excretory duct and preserve them in damar; examine with a low power (Fig. 114).

No. 92.—*Dried Tooth.*—To prepare dried ground sections of teeth they should be obtained immediately after they are extracted, sawed into transverse disks 2 mm. thick, and glued with sealing-wax upon cork and treated like No. 55. If longitudinal sections are desired the entire tooth should be glued to the cork. Longitudinal sections are to be preferred, since they show all parts of the tooth in a single preparation. If it is desired to decalcify the teeth of an adult, treat like No. 57. The enamel contains only 3 to 5 per cent. of organic substances and dissolves completely, so that only the dentine and cementum remain (Fig. 115, 116, 117).

No. 93.—*Odontoblasts.*—Remove the teeth from the jaws of a newborn child; place them in 60 c.c. of Müller's fluid; after 6 days the pulp can be easily withdrawn in toto by means of forceps. With the scissors cut from the upper surface of the pulp a piece the size of a lentil, and tease it a little in a drop of Müller's fluid; it is moderately tenacious; apply a cover-glass, press lightly upon it, and examine with the high power. At the edges of the preparation the long processes of the odontoblasts will be seen; also scattered completely isolated odontoblasts (Fig. 119). In order to preserve, treat under the cover-glass with distilled water for 2 minutes, then with picrocarmine; when the staining is completed, add dilute acidulated glycerine.

No. 94.—*Enamel Prisms.*—These may be obtained by teasing portions of the lateral surface of the teeth of No. 93 in a drop of Müller's fluid. Examine

with a high power. The enamel prisms will be found in groups of 3 and 4 and are distinguished by their dark outlines and usually indistinct cross-striation (Fig. 118). Mount in glycerine. The prismatic form of the enamel prisms may be seen in thin sections cut parallel to the surface of the teeth. Only portions of a section exhibit regular hexagonal prisms, that is, cross-sections of the prisms (Fig. 118). The enamel of younger teeth may be sectioned without previous decalcification.

No. 95.—*Development of Teeth*.—For the study of the early stages select pig and sheep embryos; these are the most easily obtained at the slaughter houses; for the first stages the pig embryos should have a size of about 6 cm., for the second stage a size of about 10 to 11 cm. For later stages the inferior maxilla of newborn dogs or cats are very suitable. Place the heads (or the lower jaws) in 100 c.c. of Kleinenberg's picrosulphuric acid, 12 to 24 hours, and harden in 80 to 120 c.c. of gradually strengthened alcohol. After the heads have lain 6 to 8 days in 90 per cent. alcohol, they are to be decalcified in 100 c.c. of distilled water plus 1 or 2 c.c. of nitric acid. When the decalcification is completed, in 3 to 8 days, harden again in alcohol. In 5 to 6 days cut off the lower jaw and divide it in front in the middle (larger jaws should be cut vertically into pieces 1 to 2 cm. long); stain the pieces in bulk in borax-carmin. When the staining and decolorization are completed, the tissue is to be transferred to absolute alcohol, in which it must remain for several days; it is then to be embedded in liver and sectioned. It is necessary to cut many (20 to 40) thick sections, since only those which pass through the middle of the tooth, or the anlage of the tooth, can be used. Mount in damar. Not infrequently in sectioning the enamel organ separates from the papilla, so that a free space exists between the two. The dentine is often stained in different tones of red; this is due to the different ages of the calcified and uncalcified strata of the dentine. The objects may also be fixed in Müller's fluid; section-staining in hematoxylin is not advisable, since too many sections must be stained which on investigation are found to be useless.

No. 96.—*Papillæ Filiformes, Fungiformes, Circumvallatæ; Follicles of the Tongue*.—Cut pieces 2 cm. square from the mucous membrane of the surface of a human tongue. Each piece should have some of the muscle tissue attached to its lower surface; for fungiform papillæ cut the piece from the tip of the tongue; for filiform, from the middle of the dorsum of the tongue; for circumvallate, from the root of the tongue, and for follicles (the punctiform openings of which can be seen with the naked eye) from the root of the tongue, and place them in 100 to 200 c.c. of Müller's fluid. The fluid must be changed several times; after 2 weeks wash the tissue and harden it in 50 c.c. of gradually strengthened alcohol. For filiform papillæ cut thick sagittal sections of the tongue and do not stain them; stain the other sections in Böhmer's hematoxylin and mount in damar (Fig. 125, 126, 127). For the preparations represented in Fig. 128 and Fig. 130 the tissue was fixed and hardened in 50 c.c. of absolute alcohol. Rabbits' tongues may be placed in toto in 200 c.c. of Müller's fluid; the subsequent treatment is the same. Thick cross-sections through the anterior half of the entire tongue are suitable for the study of the arrangement of the muscles of the tongue. Thin sections of the root of the tongue show beautiful mucous and serous glands.

No. 97.—*The Tonsils*.—The tonsils of adult man do not furnish instructive preparations. They should be treated like No. 96. The tonsils of the rabbit and the cat are to be recommended; to find these proceed as follows:—

Dissect the skin from the anterior surface of the neck and remove

the structures lying over the trachea and esophagus; with a pair of stout scissors cut through both tubes above the sternum, grasp the cut ends with forceps, and with scissors dissect them up to the head of the pharynx, keeping close to the anterior surface of the vertebral column (at the same time the cornua of the hyoid bone will be divided). Cut through the musculature close to the median edges of the inferior maxilla, and also through the ligaments of the tongue (glosso-epiglottic). (In the rabbit it is advisable to divide both angles of the mouth, and with scissors introduced within the slit to sever the ligaments and the genio-hyoglossus muscle.) Draw the trachea and attached structures downward, press the tongue down between the rami of the inferior maxilla, and divide its remaining attachments (to the palate) close to the bone. Put the tongue down with its free surface looking upward. With delicate scissors divide the posterior wall of the pharynx in the median line down to the larynx and pull the walls apart; the tonsils will then be seen as a pair of oval prominences, about 5 mm. long, on the lateral walls of the pharynx. They may be fixed in 60 c.c. of Kleinenberg's picrosulphuric acid (p. 28), and hardened in 50 c.c. of gradually strengthened alcohols (p. 29), stained with hematoxylin or with eosin and hematoxylin (p. 31), and mounted in damar.

No. 98.—*The Esophagus*.—Pieces of human esophagus 2 cm. square and of that of the rabbit and cat 2 cm. long of the entire tube are to be fixed in 60 c.c. of Müller's fluid and in 2 weeks hardened in 50 c.c. of gradually strengthened alcohol; stain with Böhmer's hematoxylin; mount in damar (Fig. 131).

No. 99.—*The Mucous Membrane of the Stomach*.—For topographical preparations place pieces 2 to 5 cm. square for 6 hours in 100 c.c. of 3 per cent. nitric acid. Remove the gastric contents adhering to the mucous membrane by moving it slowly to and fro in the acid. In a half hour renew the acid, and harden in 60 c.c. of gradually strengthened alcohol. Mount thick unstained sections in damar (Fig. 132).

No. 100.—*Fresh Gastric Glands*.—From the fundus of the stomach of a rabbit just killed cut pieces about 2 cm. square and separate the loosely-attached muscular coat from the mucous membrane. Grasp the latter with forceps at the left edge and with fine scissors cut very thin strips, 0.5 to 1 mm. thick; tease them in a drop of 0.5 salt solution. The body and fundus of the fundus glands can be satisfactorily isolated without much trouble. The bodies of the parietal-cells may be distinctly seen (Fig. 265, *B*), the chief-cells are not visible. The nuclei may be stained with picrocarmine and the preparation mounted in dilute glycerine. The isolation of the pylorus glands can only be accomplished by very careful teasing.

No. 101.—*Isolated Gastric Epithelium*.—Place pieces 1 cm. square of gastric mucous membrane for about 5 hours in 30 c.c. of Ranvier's alcohol (see further p. 26 *a*). In the majority of the cells the mucous portion occupies a large division, and they have the appearance of those pictured in Fig. 12 *c*. The preparation may be stained under the cover-glass with picrocarmine, and mounted in diluted acidulated glycerine.

No. 102.—*Gastric Glands*.—The stomach of a cat or dog that if possible has been fasting for one or two days is especially to be recommended. The stomach of the rabbit, on account of the very small size of the chief-cells, is less suitable. Dissect off the mucous membrane from the muscular coat and place pieces of the former about 1 cm. square in about 10 c.c. of absolute

alcohol. In about a half-hour transfer them to 20 c.c. of fresh alcohol. The outlines of the glands can be recognized in moderately thin sections; the only difficulty is the circumstance that the gland-tubules are placed very close together. The beginner may not recognize the glands and may mistake for them the gastric pits lined with clear epithelium. The stomach of man, which however is suitable for use only for a few hours after death, exhibits this difficulty in a less degree. For the study of the minute structure of the glands and of the superficial epithelium, embed the tissue in liver and cut the thinnest possible sections.

a. *For fundus glands, chief- and parietal-cells*, cut vertical or better horizontal sections of the mucous membrane and stain them with Böhmer's hematoxylin, 2 to 4 minutes. Wash the sections thoroughly in 30 c.c. of distilled water, which must be changed as often as it becomes bluish—about once or twice. Transfer them to 5 c.c. of a $\frac{1}{10}$ per cent. solution of Congo red (p. 23), 3 to 6 minutes, wash 2 minutes in distilled water, and mount in damar. If the sections are too thick, everything appears red; the large red parietal-cells cover the smaller chief-cells; examine the thinnest parts of the sections, especially the fundi of the glands, where the parietal-cells are not so exceedingly profuse. The parietal-cells can be recognized with the low power as isolated red spots on a rosy-red ground. With the high power the pale blue smaller chief-cells can be seen. The very narrow lumen of the fundus glands may be best seen in cross-sections of the follicle (sections parallel to the surface of the mucosa). The lateral twigs of the chief lumen can only be perceived in very favorable sections (Fig. 134). Fig. 133 is a combination of several thin longitudinal sections.

b. *For pylorus glands*, stain vertical and horizontal sections of the mucosa with Böhmer's hematoxylin and mount in damar. The lumen of the pyloric glands is wider (Fig. 136).

FIG. 265.—LOWER HALF OF AN ISOLATED FUNDUS-GLAND OF RABBIT. $\times 240$. B, Parietal-cell; M, chief-cell.

No. 103.—*Brunner's Glands*.—Cut out the stomach and duodenum of a cat about 1 hour after death. Open both along their length, remove the contents by swaying them gently to and fro in salt solution (p. 19), and place the pyloric end of the stomach and the upper half of the duodenum, that is, in all a piece 5 to 6 cm. long, for 6 hours in 100 c.c. 3 per cent. nitric acid. Further treatment like No. 99. Cut longitudinal sections, which pass simultaneously through the pylorus and duodenum. Stain with Böhmer's hematoxylin. Mount in glycerine or in damar (Fig. 136). If the tissue be placed in the acid immediately after death the smooth muscle of the intestine contracts so that a rigid curving of the intestinal wall takes place.

No. 104.—*Epithelium and Villi of the Small Intestine*.—From the small intestine of a rabbit just killed, cut a piece 1 cm. long, open it along its length and remove the contents by carefully pouring over it $\frac{3}{4}$ per cent. salt solution. Then grasp the piece at the left edge with the forceps, with fine scissors cut off a small strip, and spread it out in a drop of salt solution on a slide on a black background. With the unaided eye one can see the villi projecting from the edge of the preparation. Examine the preparation without a cover-glass, with the low power. The villi will be seen partly extended, partly contracted;

the latter condition may be recognized by transverse folds running across the villi (Fig. 266). Details cannot be detected. Apply a cover-glass; the villi thus become flattened and appear clearer; the cylindrical epithelium, and close beneath this the loops of the capillary blood-vessels, can be distinctly seen. If the epithelium contains goblet-cells, these appear as bright shining rounded spots. For the investigation of the epithelium, proceed as follows:—

a. Tease the piece a little; in this way columnar cells, singly and in groups, may be isolated, which are to be examined with the high power. Not infrequently a few columnar cells are found inflated and of a spherical form. The basal border sometimes shows very distinct rods. Goblet-cells, when present, may be recognized by their homogeneous appearance, and if carefully focused the sharply-outlined orifice may be perceived. Occasionally the epithelial cells are difficult to loosen from the basement-membrane; in such cases make a second investigation an hour later, when the epithelium will be sufficiently macerated to be brushed off.

b. For permanent preparations place pieces (1 cm. square) of the intestine in 30 c.c. of Müller's fluid. In 3 to 5 days take the tissue out, scrape it with the tip of a scalpel, and distribute a little of the scraping in a drop of diluted glycerine; cover-glass; high power (Fig. 139, A).

No. 105.—*Sections of the Small Intestine.*—Place pieces 2 to 4 cm. long of the intestine of a rabbit, better, of a puppy or a kitten, in 100 to 200 c.c. of 3 per cent. nitric acid. After 6 hours the pieces are to be hardened in 100 c.c. of gradually strengthened alcohol. Sections can be made through the entire intestinal tube; in most cases, only fragments of the villi are thus obtained; to obtain entire villi cut open the hardened intestine along its length with a razor, pin it with needles on a cork-plate, with the mucosa uppermost. The villi can then be seen with the unaided eye. Cut thick cross-sections, stain them for one minute with Böhmer's hematoxylin, and mount in damar. Goblet-cells are very frequently found in the epithelium (Fig. 139, B). Staining in bulk with borax-carminé is to be strongly recommended.

FIG. 266.—INTESTINAL VILLUS OF A RABBIT. $\times 70$.

The human intestine, before being placed in the nitric acid, must be cut open and washed in the same fluid. It is advisable to pin pieces about 5 cm. square to a cork-plate and thus to place them in the fixing and hardening fluids. If the intestine is not absolutely fresh, the entire superficial epithelium loosens so that the naked connective-tissue villi lie exposed.

Horizontal sections of the intestine furnish very beautiful pictures. Not infrequently the cross-sections of the glands drop out and then only the connective-tissue tunica propria remains. In these preparations the goblet-cells all appear as clear bodies of equal size, and therefore afford no clue in regard to the functional condition of the cell.

For the latter purpose the following is to be recommended:—

No. 106.—*Triple Staining of the Intestine.*—Small pieces of tissue are to be fixed in Flemming's mixture (p. 21), hardened in gradually strengthened alcohol, and subsequently treated according to the method given on p. 34, 10.

No. 107.—*The Patches of Peyer.*—These can be seen shimmering through the uninjured fresh intestinal wall of the rabbit, but in the dog and in the cat they are often (on account of the thickness of the muscular coat) not at all perceptible. In the latter animals patches are constant at the point

where the small intestine opens into the large. Cut out the portion of the intestine of a rabbit containing the Peyer's patches and proceed according to the method given in No. 105. In the cat take the lowermost portion of the ileum (about 2 cm. long) with a piece of the cecum of the same length; open both along their length and span them out on a cork-plate, with the mucosa uppermost. Usually the mucosa is covered with a tenaceous excrement, difficult to remove by washing, and which glues the villi together, so that only oblique sections of the villi can be obtained. Further treatment like No. 105.

Closely-placed nodules are found in the blind half of the vermiform process of the rabbit, which encroach upon the mucosa and compress it to such narrow areas that cross-sections exhibit very complicated pictures, scarcely intelligible to the beginner.

Fixation in 0.1 per cent. chromic acid and hardening in gradually strengthening alcohols renders the germinal centers very distinct, but is not so good for the remaining elements as the nitric acid.

No. 108.—*The Large Intestine*.—Treat empty pieces like No. 105 or 106 (compare with Fig. 13, p. 59). Pieces filled with feces must be cut open, washed, and spanned on cork.

No. 109.—*Fresh Crypts of the Large Intestine of the Rabbit*.—Cut a piece 1 cm. long from the lowermost portion of the large intestine (between two spherical masses of feces) place it on a dry slide, open it with the scissors, and spread it out with the mucous surface uppermost; add a drop of $\frac{3}{4}$ per cent. salt solution, grasp the piece with forceps at the left edge, and with fine scissors cut off an extremely thin strip. Transfer this with a drop of the salt solution to another slide; with needles separate the muscularis from the mucosa and tease the latter a very little; apply a cover-glass with slight pressure. With a low power the follicles of the crypts can be readily seen, but it is difficult to detect their orifices (Fig. 267). The epithelial cells are often granular in the portion bordering the lumen. With the high power the superficial epithelium can be very well seen from the side and from the surface. The contents of the goblet-cells are often not clear, as in sections, but dark and granular.

FIG. 267.—e, Epithelium; l, crypts of Lieberkühn. $\times 80$.

No. 110.—*Blood-vessels of the Stomach and the Intestines*.—A stomach and intestine injected from the descending aorta, are to be fixed in 50 to 200 c.c. of Müller's fluid and hardened in gradually strengthened alcohols. One portion should be cut into thick (up to 1 mm.) sections, stained, and mounted in damar (Fig. 144), and another part used for horizontal preparations, which with the low power and change of focus are very instructive. For this purpose pieces of the large intestine, 1 cm. square, may be transferred from absolute alcohol to 5 c.c. of turpentine for clearing, and mounted in damar. It is easy to strip the muscularis from the mucosa and to mount the separate coats in damar.

No. 111.—*Auerbach's and Meissner's Plexus*.—For this purpose the intestine of the rabbit and guinea-pig (not of the cat) are especially suitable. It is not necessary that the object be absolutely fresh; the small intestines of children several days after death can still be used. Prepare 200 c.c. of a dilute solution of acetic acid (10 drops of glacial acetic acid to 200 c.c. of distilled water). Then separate a piece (10 to 30 cm. long) of the small intestine from the mesentery. Cut it open and brush out the contents lightly with the finger;

tie the lower end of the intestine and fill it from the upper end with the diluted acetic acid; tie it above and place the whole piece in the remainder of the acetic acid. In 1 hour change the fluid. In 24 hours transfer the intestine to distilled water, with scissors open it along one side of the line of attachment of the mesentery, and cut off a piece 1 cm. long. The muscularis can be readily separated from the mucosa with the aid of forceps; they are only firmly united at the line of attachment of the mesentery.

a. Auerbach's Plexus.—If a piece of black paper be placed under the glass dish containing the tissue, the white nodal points of Auerbach's plexus can be seen by the unaided eye. Transfer a piece of the muscularis, about 1 cm. square, in a drop of the diluted acetic acid to a slide; examined with the low power it furnishes a very pretty picture (Fig. 145, A). If it is desired to preserve the preparation, place the tissue for 1 hour in 30 c.c. of distilled water, which must be changed several times, and then for from 8 to 16 hours in 5 to 10 c.c. of a 1 per cent. osmic acid solution, *in the dark*; wash the piece quickly in distilled water and mount in diluted glycerine. The osmium preparations are not as beautiful as the fresh ones in the acetic acid. In the guinea-pig both strata of the muscularis can be readily separated (if the intestine is absolutely fresh on being filled with the dilute acid); the plexus remains attached to one stratum. Pieces of this should be placed for 1 hour in distilled water, then treated with gold chloride (p. 37), and mounted in damar. The gold-chloride treatment is less adapted to human intestines, since both the muscular layers are likewise stained red and partially conceal the plexus. The firm union of the muscular strata in the human organ may be due to the age of the object.

b. Meissner's Plexus.—With a scalpel scrape the epithelium from the isolated mucosa; place a piece about 1 cm. square on a slide; apply a cover-glass, press upon it slightly, and examine with the low power (Fig. 145, B). To preserve the preparation, proceed as in No. 111, *a*; but it is advisable to span the pieces on cork and before transferring them from the absolute alcohol to the bergamot oil, to press them somewhat, in order that the alcohol may be completely removed from the spongy mucosa.

In addition to nerves, many blood-vessels are present, which may be easily recognized by the structure of their walls, in part by the transversely-placed nuclei of the muscle-fibers.

No. 112.—*The Parotid, Submaxillary, and Sublingual Glands.*—From human glands (still useful in winter after 3 or 4 days) cut a number of pieces 0.5 to 1 cm. square, and place them in 30 c.c. of absolute alcohol, which should be changed in 5 to 20 hours. In 3 days the tissues are ready to be sectioned, and can be used at once or later. Stain 1 piece in bulk in borax-carminé. Embed another in liver and cut the thinnest possible sections; small fragments about 2 mm. long can be used; stain them in Böhmer's hematoxylin, 2 to 3 minutes; the transfer of the sections to the staining solution must be done slowly, or the most delicate sections will be destroyed; then stain with eosin (p. 32), and mount in damar. (Very thin sections should be examined in water after the staining in hematoxylin is completed, since the cell boundaries are then very much more distinct.) If the staining is successful, the salivary tubules and the crescents are red. In the sublingual gland and in the mucous cells of the submaxillary the membrana propria also stains red; it must not be confused with the sections of the crescents, which latter are granular, while the membrana propria has a homogeneous appearance. The mucous cells in the borax-carminé preparations are clear throughout. In the sections stained with hematoxylin they are sometimes clear, sometimes a pale blue of different shades (Fig. 146); the portion which stains is a reticulum which occurs in

certain functional stages of each mucous cell. The very short intercalated pieces of the submaxillary gland are difficult to find; on the other hand, they may be easily seen in the parotid (also in that of the rabbit). Of the end-pieces only certain portions, those which have been accurately halved and the lumen of which is visible, are suitable for study. The numerous oblique and tangential sections are often very difficult to understand (Fig. 146, 4, 5, 6, 7).

No. 113.—*The Pancreas*.—The human pancreas as a rule cannot be used. The treatment is the same as for the parotid gland, No. 112. The characteristic granular zone of the gland-cells, bordering the lumen, is not to be seen by this method (Fig. 149, *B*). Tease a pinhead-sized piece of the fresh pancreas of a cat in a drop of $\frac{3}{4}$ per cent. salt solution. With the low power the acini appear spotted; this is due to the partly clear and partly granular divisions of the cell. With high magnification the tissue appears like Fig. 149, *A*.

No. 114.—*Liver Cells*.—Make an incision in a fresh liver and with the blade of the scalpel obliquely placed scrape the cut surface. The brown liver-tissue attached to the blade is to be transferred to a slide and a drop of salt solution added. Apply a cover-glass. Examine first with the low power then with the high (Fig. 153, *A*). The preparation contains, in addition to the liver-cells, numerous colored and colorless blood-corpuscles.

No. 115.—*Hepatic Lobules*.—Place small pieces (about 2 cm. cubes) of a pig's liver in 30 to 50 c.c. of absolute alcohol. The majority of the lobules are hexagonal; they can be seen on the surface of the liver by the unaided eye, and after a moment become distinctly visible on the cut surface. The section of the central vein also becomes visible. In about 3 days sections can be made; stain them with Böhmer's hematoxylin. The division into lobules can be well seen with the low power, but the hepatic cells as well as the bile-ducts are less satisfactory for study. Better for this purpose is the following.

No. 116.—*Human Liver*.—Place pieces about 2 cm. square, as fresh as possible, for 4 weeks in 200 c.c. of Müller's fluid for fixation and then in 100 c.c. of gradually strengthened alcohols for hardening. Examine unstained sections (parallel and also vertical to the surface) and stain others with Böhmer's hematoxylin and also with eosin; mount in damar. The demarcation of the lobules is not distinct, because of the slight development of the interlobular connective tissue. The division into lobules may be more readily perceived on macroscopic inspection, than on investigation with the microscope. For orientation the beginner should recall that isolated sections of blood-vessels always represent intralobular veins; while numbers of sections together represent branches of the portal vein, of the hepatic artery, and of the bile-duct. Exact transverse sections of central veins may also be recognized by the cords of hepatic cells radiating from them (Fig. 154). For the study of the structure of the gall-bladder as well as of the larger bile-ducts, only absolutely fresh livers can be used, since the alkaline bile permeates the walls of the gall-bladder soon after death, stains the tissue yellow, and renders it unfit for microscopic investigation.

No. 117.—To demonstrate the *capillaries* and the *intralobular connective tissue*, which in ordinary preparations are scarcely visible, shake a number of thin double-stained sections of human liver (No. 116) for from 2 to 3 minutes in a test-tube half filled with distilled water. The liver-cells in part fall out; the edges of the preparation are then to be examined in a drop of water (Fig. 163). This preparation can be mounted in damar, but the more delicate connective-tissue fibers disappear therein.

No. 118.—*Blood-Vessels of the Liver.*

a. Chloroform a rabbit and quickly place a 2 cm. cube of the liver (without allowing much blood to flow from it) in 50 c.c. of absolute alcohol. In 2 days the natural injection can be seen on the surface; it is indicated by brown spots within the centers of the lobules. Cut thick sections parallel to the surface, and mount them unstained in damar. Examine with a low power. Very frequently only the superficial strata of the liver contain filled blood-vessels.

b. Of all injections that of the liver is most easily accomplished. Inject Berlin blue (p. 37), either through the portal vein or the inferior vena cava; in the latter case it is advisable to make an incision above the diaphragm, to allow the heart to rest upon it, and to insert the canula through the right auricle into the inferior cava. The injected liver is to be placed in toto in about 500 c.c. of Müller's fluid; after 6 days pieces about 2 cm. square of the portions best injected are to be cut out, placed again for 2 to 3 weeks in about 150 c.c. of Müller's fluid, and finally hardened in 100 c.c. of gradually strengthened alcohols. Cut thick sections and mount them unstained in damar (Fig. 157, 158, 159).

No. 119.—*Exhibition of Gland Lumina by Golgi's "Black Reaction."*—

Place small pieces of stomach, of salivary glands, and of liver for 3 days in the osmio-bichromate mixture (in winter, in the warm chamber, p. 36), and for the same length of time in the silver solution. For further treatment see p. 35. Very often the staining does not succeed until after the procedure has been repeated once or twice. After-staining (p. 37) is to be advised. In the liver the "lattice-fibers" stain occasionally.

No. 120.—*The Endothelium of the Peritoneum.*—Proceed as in No. 35, but instead of taking the mesentery, which also yields instructive pictures, use the greater omentum. The pieces may be stained in Böhmer's hematoxylin and mounted in damar (Fig. 167).

No. 121.—*The Connective-tissue Reticulum.*—This may be obtained by spreading out a fragment of a fresh human omentum in a few drops of picrocarmine. Mount in diluted glycerine (not acidulated).

XIII. THE RESPIRATORY ORGANS.

No. 122.—*The Larynx, the Bronchi, and the Thyroid Gland.*—Of animals, the cat is especially suitable. Expose the bronchi above the manubrium, cut them and the esophagus through transversely and dissect both loose upwards (see No. 97). The tongue may be removed with these parts. The thyroid gland should be allowed to remain attached to the larynx. The whole is to be placed for from 2 to 6 weeks in 200 to 400 c.c. of Müller's fluid, then washed for 1 hour in running water and hardened in 200 c.c. of gradually strengthened alcohol. In about 8 days cut sections, transverse and longitudinal, through the vocal cords and portions of the trachea; stain them for 5 minutes in Böhmer's hematoxylin, and mount in damar. Especially instructive are sections taken transversely through the vocal cords, in which the mucous membrane, glands, muscles, blood-vessels, nerves, and cartilage furnish material for the most varied study.

No. 123.—*The Bronchi.*—From an animal just killed (rabbit) remove the lungs, fix them in Müller's fluid and harden them in gradually strengthened alcohol, like No. 122. In 8 days cut out of the lung 1 cm. cubes, which

contain a portion of a longitudinally-disposed bronchus. With the scissors remove the greater part of the attached lung tissue; embed the bronchus in liver, and make thin transverse sections, which may be stained in Böhmer's hematoxylin and mounted in damar (Fig. 168). The lungs of cats are less suitable than those of the rabbit, owing to the often considerable masses of fat surrounding the bronchi. This method is also applicable for the exhibition of the alveoli and the alveolar passages.

No. 124.—*The Respiratory Epithelium*.—For the demonstration of this tissue only animals just killed can be used. Young kittens (*not* newborn) are suitable; they should be killed by decapitation. The trachea and lungs should then be carefully taken out and filled by means of a glass pipet with a previously-prepared solution of silver nitrate (50 c.c. of a 1 per cent. solution to 200 c.c. of distilled water). The trachea should then be tied fast and the whole placed (1 to 12 hours) in the remainder of the silver solution and stood in the dark. On removing them from the silver solution, the lungs should be quickly washed with distilled water and transferred to 150 c.c. of gradually strengthened alcohol, in which they may remain (in the dark) for an indefinite length of time. The reduction can be undertaken in an hour after the silver injection, or later. For this purpose the lungs in the alcohol should be exposed to sunlight, in which they become a deep brown in a few minutes. With a *very sharp* razor cut thin sections, taking care not to compress the tissue. Despite the hardening in alcohol the lung tissue is still soft and allows only thick sections to be cut. Sections may be most easily cut in a direction parallel to the surface. Place the sections for from 10 to 60 minutes in 5 to 10 c.c. of distilled water to which a crystal of common salt about the size of a lentil has been added, and then mount them unstained in damar. It is not advisable to employ nuclear staining, since not only the nuclei of the epithelial cells, but also those of the capillaries and other tissues are colored, and consequently the picture becomes very complicated. Orientation in such sections is not altogether easy. The investigation should be begun with the low power. The small alveoli are easily recognized; the somewhat larger spaces correspond to alveolar ducts. The demarcation of the epithelium is on the whole finer with medium magnification (80 diameters), and by no means equally good in all places. The cubical epithelial cells are usually colored a somewhat deeper brown. Find a good place, study it with the high power (240 diameters), and by changing the focus (elevating and depressing the tube) note the relief of the preparation; with high magnification, either only the base or the edge of an alveolus can be distinctly seen. Fig. 170 was drawn with change of focus.

No. 125.—*Elastic Fibers of the Lungs*.—With the scissors placed flatwise on the lung (the lung need not be fresh), cut a flat piece 1 cm. square, spread it out with needles on a dry slide, apply a cover-glass and treat with 2 drops of potash lye diluted 1_2 with water; the diluted lye destroys all parts excepting only the elastic fibers, whose thickness and arrangement may be easily investigated with the high power (240 diameters).

No. 126.—*Blood-vessels of the Lungs*.—Inject the lung from the pulmonary artery with Berlin blue; fix it in Müller's fluid, and harden it in alcohol. Cut thick sections, principally parallel to the surface of the lung (Fig. 171).

No. 127.—*The Thyroid Gland*.—Thin sections of the gland, hardened in toto (see No. 122), are to be stained with picrocarmine and mounted in damar (Fig. 172). The retracted colloid masses stain an intense yellow. Examine thick sections in glycerine, in which the lymph-vessels filled with colloid substance are often distinctly visible.

No. 128.—*The Thymus Body*.—Place the thymus body of a young animal, for from 2 to 5 weeks, in Müller's fluid and harden it in gradually strengthened alcohol. Stain sections with Böhmer's hematoxylin; mount them in damar (Fig. 173). Care should be taken not to confuse the cross-sections of the blood-vessels, the lumina of which change in elevating and depressing the tube (when they are not true cross-sections), with the concentrically-striated corpuscles of Hassall. The preparation represented in Fig. 174 is from a thymus body fixed in Flemming's mixture and stained with saffranin.

XIV. THE URINARY ORGANS.

No. 129.—*Isolated Uriniferous Tubules*.—The most suitable for this purpose are the kidneys of young animals, for example newborn kittens. Divide the kidney in halves; place one half (*a*) aside for investigation fresh; cut the other half (*b*) into pieces including the cortex and medulla, and place them in 30 c.c. of pure hydrochloric acid.

a. Tease a pea-sized piece in a drop of 0.75 per cent. salt solution. The red glomeruli, the convoluted and straight uriniferous tubules, can be seen with the low power. The convoluted tubules are dark and granular, the other divisions clear. With high magnification, the nuclei of the clear portion of the uriniferous tubules can be distinctly seen; the cell boundaries may best be seen in the collecting tubules. In the convoluted tubules only the fine striation of the bases of the gland-cells can be seen; cell boundaries and nuclei are not visible.

b. In about 2 hours the red pieces of kidney tissue should be transferred to a capsule containing 50 c.c. of distilled water, in which they rapidly turn a dirty gray and acquire smeary surfaces. The water is to be changed. After a few moments small pieces can be detached with needles and readily separated into tubules, in a little water on a slide. If it is desired to obtain entire uriniferous tubules, transfer pieces of kidney 2 cm. square to a watch-glass in which has been placed a cover-glass and enough distilled water to cover the surface of the latter. The tubules should now be isolated with needles. If the isolation is successful—this may be ascertained by examination with low power—carefully absorb with filter-paper the water from the watch-glass and then from the cover-glass, take out the latter, cleanse its free surface, and place it with the attached tubules gently on a slide on which a drop of dilute glycerine has been previously placed. The preparation may be subsequently stained under the cover-glass with picrocarmine (Fig. 176).

No. 130.—*The Cortex and Medulla*.—For sections the kitten's other kidney or other pieces of kidney tissue 2 to 3 cm. square are to be fixed in 200 to 300 c.c. of Müller's fluid, and in 4 weeks hardened in 100 c.c. of gradually strengthened alcohol. Fixation in absolute alcohol (like No. 132) is still better. Thick transverse and longitudinal sections through the cortex and similar ones through the medulla are to be examined unstained in dilute glycerine, with a low power. Thin transverse sections through the apex of the papillæ, through the base of the papillæ for the excretory duct (Fig. 181), and through the cortex are to be stained with Böhmer's hematoxylin and mounted in damar. Endeavor to cut sections through the cortex and the medulla, showing the boundary between the two; examine them unstained in glycerine, with the low power. Frequently the blood-vessels are still filled with blood-corpuscles and may be traced for long distances.

No. 131.—*Medullary rays and Henle's loops* are especially fine in stained vertical sections of the kidneys of young animals treated like No. 130.

No. 132.—For the study of the *glomeruli and Bowman's capsule*, also the connection of the latter with the uriniferous tubule, the kidney of the mouse is most suitable. Fix and harden the divided kidney in 15 c.c. of absolute alcohol, which should be changed in an hour. After 3 days (or later) cut thin sections of the cortex, stain 2 to 3 minutes in Böhmer's hematoxylin, and mount in damar (Fig. 179). The invaginated portion of the capsule, on account of the similarly-stained nuclei of the blood-vessel walls, cannot be distinguished. The appearance of the cells in the varying phases of secretion can only be investigated in the absolutely fresh object fixed in Flemming's mixture (p. 21), and cut in very thin sections.

No. 133.—*The Blood-vessels of the Kidney*.—An isolated kidney may be injected (p. 37) and fixed in 300 c.c. of Müller's fluid for 4 weeks and then hardened in 150 c.c. of gradually strengthened alcohol. The venæ stellatæ can be investigated macroscopically. Unstained thick longitudinal and transverse sections should be studied with the low power (Fig. 182).

No. 134.—*Nerves of the Kidney*.—Treat small pieces according to the method given on p. 35; they should remain 3 to 6 days in the osmio-bichromate mixture.

No. 135.—*The Pelvis of the Kidney and Ureters*.—Of the former pieces 1 cm. square, of the latter 1 to 2 cm. long should be fixed in Müller's fluid, and in 14 days hardened in 100 c.c. of gradually strengthened alcohol. Stain sections with Böhmer's hematoxylin and mount in damar.

No. 136.—Treat the *Bladder* like No. 135.

No. 137.—*Epithelial Cells of the Pelvis of the Kidney, the Ureter, and the Bladder*.—Place pieces of these parts, 1 cm. square (cut open the ureter), in 30 c.c. of Ranvier's alcohol. Isolate and stain with picrocarmine. Mount in diluted acidulated glycerine.

No. 138.—*The Female Urethra*.—Cut out a piece of the female urethra about 2 cm. long, together with the attached anterior vaginal wall; place it in 100 to 200 c.c. of Müller's fluid for fixation, and in 2 to 3 weeks harden it in gradually strengthened alcohol (p. 29). Stain cross-sections in Böhmer's hematoxylin (p. 31) and mount in damar (p. 38).

No. 139.—*The Male Urethra*.—Treat pieces 1 to 3 cm. long of the prostatic, membranous, and cavernous portions and of the fossa navicularis like No. 138. Care should be exercised not to confuse the lacunæ of Morgagni (blind evaginations of the mucosa) with sections of glands.

XV. THE TESTICLE AND THE OVARY.

No. 140.—For a general view of the testicle make a transverse incision through the testicle and epididymis of a newborn child; fix the pieces in about 50 c.c. of Kleinenberg's picrosulphuric acid (p. 21) and harden in 30 c.c. of gradually strengthened alcohol (p. 29). Stain thick transverse sections of the entire organ in dilute carmine (p. 32), and in Böhmer's hematoxylin (p. 31), and mount in damar. Examine with very low magnification (Fig. 186). In the testicle of the rabbit, cat, and dog the corpus Highmori is not at the margin but in the center of the organ. If no incision is made into the organ, it does not harden sufficiently, because the dense tunica albuginea retards the penetration of the fluids.

No. 141.—*Minute Structure of the Seminiferous Tubules*.—Place small pieces (2 cm. cubes) of the fresh testicle of an ox in 200 c.c. of Müller's fluid (p. 14), and after 14 days harden them in 50 c.c. of gradually strengthened alcohols. Cut sections as thin as possible, stain them in Böhmer's hematoxylin (p. 31), and mount in damar (p. 38). Even with the low power tubules in a condition of activity can be distinguished from resting tubules; the former may be recognized by the intensely blue heads of the young spermatozoa (Fig. 187).

No. 142.—Still better preparations may be obtained by placing the entire testicle of a mouse in 10 c.c. of the platino-aceto-osmic acid mixture (p. 21) for 24 hours for fixation, then washing it for several hours in running water, and hardening it in 20 c.c. of gradually strengthened alcohols. Mount the unstained sections in damar (Fig. 188). The platino-aceto-osmium mixture does not penetrate sufficiently into the testicles of larger animals, which therefore are not suitable.

No. 143.—*Elements of the Testicle*.—Place pieces about 1 cm. in size of the fresh testicle of an ox in 20 c.c. of Ranvier's alcohol (p. 19) and in 5 to 6 hours tease the tubules in a drop of the same alcohol. Stain under the cover-glass with picrocarmine, and mount in dilute glycerine. Several preparations from different parts of the organ should be completed, and then not infrequently the cells of Sertoli with attached spermatocytes, or the seminal filaments produced by them, will be obtained (Fig. 268, *b*).

FIG. 268.—ISOLATED ELEMENTS OF TESTICLE OF OX. $\times 240$. *a, c*, Mother-cells; *b*, "spermatoblast;" *d*, Immature seminal filament; *e*, mature seminal filament.

No. 144.—*Elements of the Semen*.—Make an incision into a fresh epididymis, and place one drop of the milk-white fluid that exudes from the cut surface on a clean slide; add one drop of salt solution, apply a cover-glass, and examine with the high power. After a time let one drop of distilled water flow under the cover-glass; the movements of the spermatozoa soon cease; the heads of the majority of the seminal filaments then present their broad surface, and the tail curves and forms a loop (Fig. 189, 3). Remnants of protoplasm still adhere to seminal filaments not fully matured. The spermatozoa may be preserved by allowing the semen diluted with water to dry on the slide; then apply a cover-glass and secure it with cement (p. 38). In examining such preparations, too much illumination gives rise to troublesome reflections. For spiral fibrils, examine the spermatozoa of the rat in water; use an immersion lens.

No. 145.—The vitality of the seminal filaments has led to *investigations for forensic purposes*. It may, for example, be a question as to whether spots occurring on a linen garment were produced by semen. Cut strips 5 to 10 mm. long from the suspected spots, soak them for from 5 to 10 minutes in a watch-glass containing distilled water, and tease a few fibers. With the high power (500:1) examine chiefly the edges of the isolated linen fibers, to which the seminal filaments if present are attached. Not infrequently the heads have been broken off; they are recognized by their peculiar luster, their shape, and their (in man small) size.

No. 146.—*Seminal Filaments of the Frog*.—The male frog is recognized by a well-developed wart on the ball of the thumbs. Open the abdominal cavity; the testicles are a pair of oval bodies (similar to the kidneys of mam-

mals, lying to either side of the vertebral column. Divide the organ by a transverse incision; dilute a drop of the fluid with a drop of salt solution. The seminal filaments are large, the head thin and elongated, the tail so delicate that at the first glance it may be overlooked. Immature filaments lie grouped in tufts.

No. 147.—*Epididymis, Vas Deferens, and Seminal Vesicles*.—Pieces 1 to 2 cm. in size are to be fixed in 200 c.c. of Müller's fluid and in 14 days hardened in 60 c.c. of gradually strengthened alcohol (p. 29). Stain the sections with Böhmer's hematoxylin and mount in damar (Fig. 191 and 192).

No. 148.—The *prostate* and the different divisions of the male urethra are to be prepared in 2 to 3 cm. cubes like No. 147 (Fig. 193).

No. 149.—*The Ovary*.—The ovaries of small animals may be fixed in toto and those of larger animals with several incisions transverse to the long axis in 100 to 200 c.c. of Kleinenberg's picrosulphuric acid (p. 28), and hardened in 100 c.c. of gradually strengthened alcohol (p. 29). For a topographical view (Fig. 194) it is advisable to cut thick sections, because otherwise the contents of the follicles easily fall out. Not every section includes large follicles; it is often necessary to cut many sections in order to strike a favorable place. Stain the sections with Böhmer's hematoxylin, or in bulk with borax-carmin (p. 32). Mount in damar (p. 38).

No. 150.—Fresh *ova* may be obtained as follows: Procure the fresh ovaries of a cow. The large Graafian follicles are transparent, pea-sized vesicles, which with scissors may be easily shelled out in toto. Transfer the isolated follicle to a slide and prick it with a needle. The needle must be thrust in carefully on the side of the follicle lying against the slide, otherwise the liquor will spurt out and carry the ovum with it. With the low power, and without placing a cover-glass on the preparation, search for the ovum, which, surrounded by the cells of the cumulus ovigerus, will be found in the escaping liquor folliculi (Fig. 198, A). Place two narrow strips of paper on either side of the ovum, carefully apply a cover-glass, and examine with the high power.

Often the ovum does not escape when the follicle is pricked; it may then be found by teasing the follicle.

No. 151.—*Ova of the Frog*.—Place a small piece of the fresh ovary of a frog on a slide, and prick all the large pigmented ova, so that their contents escape. Place that which remains in a watch-glass with distilled water and wash it by moving it to and fro with needles. Place the watch-glass on a black background; the smaller, still unpigmented follicles can then be seen. Transfer the washed object to a clean slide, apply a cover-glass, and examine it. The ova have very large germinal vesicles; the germinal spot disappears early, and usually is not to be seen. On the other hand, a dark spot occurs in the vitellus, the "nucleus of the vitellus." Surrounding the ovum is a finely-striated membrane, the inner surface of which is covered with flat cells; this is the theca folliculi with the simple follicular epithelium.

No. 152.—*The Oviducts*.—Pieces 1 to 2 cm. long are to be fixed in 50 c.c. of 3 per cent. nitric acid, and after 5 hours hardened in 60 c.c. of gradually strengthened alcohol. Stain with Böhmer's hematoxylin and mount in damar.

No. 153.—*The Uterus*.—The human uterus in many cases is not suitable for the production of topographical preparations. Insurmountable difficulties are often encountered, especially in rendering the gland-tubules evident. In the two-horned uterus of many animals, the often greatly convoluted follicles

may be more readily seen; the arrangement of the muscular strata is more regular, and different from that of the human organ.

The specimens are to be prepared like No. 152.

XVI. THE SKIN AND ITS APPENDAGES.

No. 154.—*Strata of the Skin: Coil-glands*.—Cut from the pad of the finger, from the palm of the hand, or the sole of the foot, pieces of skin 1 to 2 cm. square together with a thin stratum of subjacent fat and place them in 30 c.c. of absolute alcohol. To prevent curling of the pieces pin them on a small cork-plate with the epidermis turned toward the cork, and place the whole in absolute alcohol. On the following day remove the pieces from the cork-plate and place them for from 3 to 4 weeks in 50 c.c. of 90 per cent. alcohol. Cut thin and thick sections. The latter are indispensable in order to see the excretory ducts of the coil-glands in their entire length. The most suitable for this purpose is the skin of the sole of the foot of children, because the ducts of the coil-glands here run vertically (Fig. 208). Stain with alum carmine, 10 minutes (p. 32); the red coils can be seen with the unaided eye; mount in damar. Examine with the low power. In thick sections the papillæ are often indistinct, because they are surrounded by the red colored stratum mucosum; the screw-like twisted ends of the excretory ducts may be most distinctly seen when the object is faintly illuminated or with oblique illumination (see p. 43, remark*). To render the stratum granulosum visible, bulk staining with borax-carminé, 2 to 3 days (p. 32), is to be recommended. The granules of this stratum are then stained an intense red.

No. 155.—For preparations of the *nails* fix the distal phalanx of a child 8 to 12 years of age (in adults, that of the little finger, if possible of women), 2 to 4 weeks in 100 to 200 c.c. of Müller's fluid, and harden in about 100 c.c. of gradually strengthened alcohol; decalcify (p. 29); harden again, and stain thick cross-sections 10 minutes in alum carmine (p. 32). In cutting sections place the knife on the volar side (not on the nail side) of the phalanx. The substance of the nail frequently shows differently-colored strata. In the nails of old cadavers the epithelium often becomes loosened from the ridges.

No. 156.—*Elements of the Nails*.—Place pieces of cut nail 1 to 2 mm. broad in a test-tube containing 5 c.c. of concentrated potash-lye and heat over a flame until it boils up once. Transfer the nail with a drop of the lye to a slide and scrape off some of the softened surface; apply a cover-glass. On examination with a high power, cells will be found like those in Fig. 211. For comparison, investigate the horny cells of the stratum corneum, which may be obtained by lightly scraping the pad of the finger with the handle of a scalpel. Examine the polygonal scales in a drop of distilled water, with a high power.

No. 157.—*Hairs*.—Place a hair in a drop of salt solution on a slide and examine it with the low and the high power; the most suitable for study are white hairs and the hairs of the beard. The hair-cuticle of man is very delicate and the transverse markings produced by the imbrication of the cells are often very indistinct; usually only fine wavy lines are visible. The hairs of many animals, on the other hand, show the cuticula very well, for example, sheep's wool.

No. 158.—For the demonstration of the *elements of the hairs*, place a piece of hair 1 to 2 cm. long in a drop of pure sulphuric acid on a slide and apply a cover-glass; press lightly on the glass with a needle and the cortical substance will split up into fibers, which consist of adherent cortical cells.

Slightly warm the slide, press again with a needle, so that the cover-glass becomes slightly displaced; numerous free elements, superficial scales, and cortical cells will then be seen.

No. 159.—For the exhibition of the *elements of the hair-follicles* (and the *hairs*) cut from a mustachioed human upper lip a piece 2 cm. square and place it in dilute acetic acid (5 c.c. of acetic acid to 100 c.c. of distilled water). In 2 days the individual hairs with their sheaths can be easily withdrawn and their elements separated by teasing in a drop of distilled water (Fig. 213). The cells of Henle's sheath float in small complexes in the preparation and closely resemble fenestrated membranes (Fig. 213, 5). Not infrequently a hair-follicle is obtained at the base of which a new hair is developing (compare with Fig. 126).

No. 160.—For the study of *hair and hair-follicles* place pieces 2 to 3 cm. square of the fresh skin of the scalp in about 200 c.c. of a 2.5 per cent. solution of potassium bichromate (p. 20, 9) for from 4 to 8 weeks; wash them 1 to 3 hours in running water and harden in the dark in about 100 c.c. of gradually strengthened alcohol. Longitudinal sections which include the entire length of the follicle are very difficult to cut. Macroscopic orientation as to the direction of the hair is first necessary. To obtain preparations like that in Fig. 212 thick sections, unstained, are to be mounted in glycerine. Thin sections usually include only a portion of the hair-follicle. It is much easier to cut thin cross-sections, but care must be taken to make the cut *vertical to the longitudinal direction of the hair*, not parallel to the surface of the skin. In this way a *single* section shows different levels of the hairs and hair-follicles; such sections are to be stained in dilute carmine (p. 32), and Böhmer's hematoxylin (p. 31), or better, first with hematoxylin and then with picrocarmine (p. 33) 10 minutes, and mounted in damar. Especially instructive are the sections through the hair-follicle close to the hair-bulb (Fig. 214).

No. 161.—For the *development of hair* cut pieces about 2 cm. square of the skin of the forehead (not of the hairy scalp) of a 5- to 6-months'-old human embryo; span them out (see No. 154); place them for 14 days in 100 to 200 c.c. of Müller's fluid and harden in about 100 c.c. of gradually strengthened alcohol. Stain the tissue in bulk in borax-carmine (p. 32). The sections may also be stained in Böhmer's hematoxylin (p. 31). Embed the tissue in liver; endeavor to cut sections exactly in the direction of the hair-follicle, which is much more easily done than in the hairy scalp of the adult. Mount in damar. The sections exhibit all stages of development (Fig. 215). The epidermal thickenings are only to be seen in well-preserved epidermis, which in embryos is often somewhat macerated. They are more easily found in embryos of the lower animals.

No. 162.—*Shedding and Replacement of Hair*.—The eyelids of newborn children are most suitable. Treat like No. 182. Cut sagittal sections. Vertical sections of the hairy scalp often yield good results (Fig. 216).

No. 163.—*The Sebaceous Glands*.—Fix and harden the *alæ nasi* of an infant in 100 c.c. of 2.5 per cent. solution of potassium bichromate (like No. 160). Cut thick and thin sections; stain them with dilute carmine (p. 32), and with Böhmer's hematoxylin (p. 31), and mount in damar. Sections lengthwise to the dorsum of the nose often show both sebaceous glands and hair-follicles, but they must be made exactly vertical. The *alæ* of the nose of adults, on account of the very large sebaceous glands with their wide excretory ducts, do not furnish good microscopic specimens. Small seba-

aceous glands with hair-follicles can be seen with the unaided eye in stripping off the macerated epidermis of old cadavers.

No. 164.—*Blood-vessels of the Skin*.—Inject with Berlin blue the entire hand of a child through the ulnar artery (or a foot through the posterior tibial artery) and place it in 1 to 2 liters of Müller's fluid; after several days cut pieces 2 to 3 cm. square of the palm of the hand or of the sole of the foot, place them (2 to 4 weeks) in 100 to 200 c.c. of Müller's fluid for fixation, and harden them in 100 c.c. of gradually strengthened alcohol. Cut thick sections and mount them, unstained, in damar. The papillæ in such sections are only to be recognized by the capillary loops. To the beginner it appears as if the loops extend into the stratum mucosum.

No. 165.—*For a general view of the mammary glands* place the nipple and a portion of the gland (3 to 4 cm. square) in 60 to 100 c.c. of absolute alcohol. If possible, obtain the glands of an individual that was pregnant not too long a time before; also the glands of virgins, etc. Make vertical sections through the nipple and in any direction through the gland-substance, and stain them with Böhmer's hematoxylin; mount in damar.

No. 166.—*For the minute structure of the mammary glands* place the warm living tissue (3 to 5 mm. cubes) of a pregnant mammal in 5 c.c. of Flemming's mixture (p. 21), and harden after 1 to 2 days in 30 c.c. of gradually strengthened alcohol. Cut very thin sections, stain them with saffranin (p. 32, 4), and mount in damar (Fig. 219). The structure is often difficult to understand on account of the small size of the gland-cells (in the rabbit).

No. 167.—*Elements of Milk*.—Put a drop of salt solution on a clean slide, and add to it a drop of milk. The milk is to be obtained by placing the cover-glass upon the nipple and then pressing out a drop. Examine with a high power (Fig. 221).

No. 168.—*Elements of the Colostrum*.—Proceed as in No. 167. Be careful to avoid pressure on cover-glass. The nuclei of the colostrum corpuscles can rarely be distinctly seen without further treatment; on the addition of a drop of picrocarmine they appear as dull-red spots.

VII. THE EYE AND ITS APPENDAGES.

No. 169.—Carefully cut the fresh *eye-ball* out of the optic cavity, and secure as much as possible of the optic nerve; then with the scissors cut off the attached fat and muscle, and with a *sharp* razor make an incision at the equator, about 1 cm. long, through all the coats of the eye. Place the eye-ball in 150 c.c. of 0.05 per cent. chromic acid solution (p. 20); after 12 to 20 hours, beginning at the incision already made, divide the eye-ball with the scissors completely into an anterior and posterior half, and change the fluid. After 12 to 20 hours more, wash the pieces and harden them in 100 c.c. of gradually strengthened alcohol.

a. Carefully remove the lens from the anterior half of the eye-ball and treat it further like No. 179; then cut out a quadrant and with the attached ciliary body and iris embed it in liver and cut sections through the *iridocorneal angle*. The *thick* sections are to be stained with Böhmer's hematoxylin and mounted in damar (Fig. 227).

b. From the remaining three-fourths of the anterior half of the eye-ball cut out a piece of the cornea, 5 to 10 mm. square, embed it in liver and make sections through the *layers of the cornea* (Fig. 222). The alternating lamellæ of

the substantia propria can only be well seen in unstained sections mounted in dilute glycerine.

c. From the posterior half of the eye-ball cut pieces including the three coats, 5 to 10 mm. square, and cut sections, not too thin, for the study of the *strata of the sclera and choroid* (Fig. 225). Stain them with Böhmer's hematoxylin and mount in damar. In sectioning, the retina usually becomes loosened.

d. For preparations showing the *entrance of the optic-nerve* cut around the point of entrance at a distance of about 5 mm. from the same through all the coats of the eye; embed this portion with about 1 cm. of the optic-nerve in liver and cut sections (not too thin). Place the knife so that it strikes the retina first, then the choroid and sclera, and passes through the optic-nerve longitudinally; stain with dilute carmine (p. 32) and with Böhmer's hematoxylin (p. 31), and mount in damar. Examine with very low magnification (Fig. 235).

No. 170.—Remove a fresh eye-ball according to the method given in No. 169; make an incision at the equator, and place it in 100 to 200 c.c. of Müller's fluid. In 12 to 20 hours divide it with the scissors into an anterior and posterior half. In 2 to 3 weeks carefully wash both halves in slowly running water for from 1 to 2 hours. Then cut pieces including all the coats, about 8 mm. long, and use for them the following preparations:—

a. *Teased Preparation of the Choroid*.—Tease and mount a fragment in a drop of dilute glycerine; it exhibits large blood-vessels, the capillaries of the choriocapillaris, branched pigment-cells, elastic fibers, sometimes also the glassy membrane; the "lattice-work" of the latter is only partially distinct. The isolated membranes may be stained with Böhmer's hematoxylin and mounted in damar, but the more delicate structures are thus rendered indistinct (Fig. 226).

b. *Elements of the Retina*.—Tease a small piece of the retina in a drop of Müller's fluid, carefully, with needles. Along with many fragments of the elements, a few more or less well-preserved parts will be found. Human eyes have very large, beautiful cone-visual cells, while those of many mammals are very small; wholly unsuitable in this respect are the eyes of the rabbit; unfortunately, human eyes are usually no longer in a sufficiently fresh condition when the investigation is made. The outer segments of the cones, also of the rods, are extremely delicate and rapidly disintegrate after death, falling into transverse plates and at the same time curving like a shepherd's crook. Later they disappear entirely. In order to see beautiful cone-visual cells, examine, according to the method just given, the eyes of fishes. (See further No. 171 and 172.)

c. The remaining parts of the eye-ball are to be transferred from the water to 80 c.c. of gradually strengthened alcohol for hardening; when the hardening is completed, cut out the iris, embed it in liver, and make meridional sections; stain them in Böhmer's hematoxylin and mount in damar (Fig. 228).

d. Cut out a portion 1 cm. long of the retina, including the *ora serrata*, which is macroscopically visible as a wavy line, embed it in liver, and make meridional sections; stain them in hematoxylin and mount in damar (Fig. 234).

e. Treat in the same manner a piece of the *retina* taken from the posterior portion of the eye, where the optic-fiber stratum is thickest. The radial fibers of Müller can only be seen in their entire length in accurate vertical sections (Fig. 229 and Fig. 230).

f. In the same manner treat meridional sections through the *macula* and

fovea. It is not difficult to cut sections of the macula, but on the other hand very difficult to obtain satisfactory sections through the extremely delicate fovea. The retina should not be loosened from the choroid, but the two should be sectioned together. (Among the lower mammals only the ape possesses a yellow macula and a central fovea; on the other hand, the majority—insectivora and certain rodents excepted—have an “area centralis,” without yellow pigmentation, but similar in structure to the macula. A simple or multiple fovea is always present in birds and reptiles; a fovea has also been found in bony fishes.)

No. 171.—*Fresh Elements of the Retina*.—Select the warm eyes of animals just killed. Divide the eye-ball at the equator and carefully remove the vitreous body from the posterior half; cut small pieces about 3 mm. square from the transparent retina and tease in a drop of the vitreous humor; place two thin strips of paper on either side of the preparation (p. 41), and apply a cover-glass. Isolated elements will be found only here and there; on the other hand, very good surface views are not infrequently obtained in which the rods and cones are perceptible in optical cross-section, the first as small, the latter as large circles. If at the same time a little piece of the pigmented epithelium has been transferred to the slide, the regular hexagonal cells of the same can be plainly seen with the low power. The light spots in these cells are their nuclei (Fig. 8). These cells are also very unstable and soon lose their sharp contours; molecular motion of the pigment-granules may be very frequently observed.

No. 172.—The best method for isolating the *elements of the retina* is the following: Place the eye unopened, but freed from fat and muscle, in 1 per cent. osmium solution. In 24 hours cut the eye open at the equator and place it for maceration for 2 to 3 days in distilled water; then with scissors cut out a piece of the retina about 2 mm. long and tease it in a drop of water; the preparation may be stained with picrocarmine, under the cover-glass, and mounted in dilute glycerine. With the high power, in addition to many fragments whose source is not always to be determined with certainty, elements like those pictured in Fig. 232 may be found.

It is advisable to select the eyes of small animals—*e. g.*, a small salamander (*Triton teniatus*), whose sclera is thin and allows the osmium solution to penetrate easily. For such an eye 1 to 2 c.c. of the solution will be sufficient. The form of the rods is quite different from those of mammals; they are thick and are provided with long outer segments; the cones are small.

No. 173.—*Corneal Spaces and Canaliculi*.—Select an eye as fresh as possible; of the eyes of animals, that of the ox is most suitable; with the handle of a scalpel scrape away the epithelium of the cornea; spray the denuded surface with distilled water; cut the eye through in front of the attachment of the ocular muscles and place the anterior segment, containing the entire cornea, down on the epithelial side; then with forceps and scalpel remove the ciliary body, the lens, and the iris, so that only the anterior portion of the sclera and cornea remain, which are to be placed in 40 c.c. of a 1 per cent. solution of silver nitrate. The whole is then to be placed in the dark, 3 to 6 hours, and then transferred to 50 c.c. of distilled water and exposed to sunlight (see further p. 35). Harden the objects in 50 c.c. of gradually strengthened alcohol and cut horizontal sections, which is most easily done if the cornea is held over the left index-finger. It is best to take the sections on the posterior surface of the cornea, since the spaces and canaliculi are more regular there. The sections may be stained in Böhmer's hematoxylin and mounted in damar. The pictures are negative, the spaces and canaliculi white on a brown or brown-yellow

surface (Fig. 223). Examine carefully the usually somewhat thinner margins of the section; in sections stained in hematoxylin the nuclei of the fixed corneal corpuscles are a dull blue; the contours of the cells can seldom be perceived.

No. 174.—*Fixed Corneal Corpuscles by the Gold Method.*—The method described on p. 35 is to be somewhat modified, as follows: Express the juice from a fresh lemon; filter it through flannel. Kill the animal, cut out the cornea and place it for 5 minutes in the lemon-juice, in which it becomes transparent; then wash it in 5 c.c. of distilled water for 1 minute; transfer it to 10 c.c. of gold-chloride solution and place it in the dark for 15 minutes. With glass rods transfer the cornea to 10 c.c. of distilled water for 1 minute, then to 50 c.c. of distilled water to which 2 drops of acetic acid have been added, and expose it to daylight; in 24 to 48 hours the reduction is completed. The object is then to be placed in 10 c.c. of 70 per cent. alcohol (in the dark); on the following day cut out a little piece of the cornea, hold it with needle and scalpel at the edges and separate the thin lamellæ from the posterior surface; this can be done successfully without much trouble. Mount the lamellæ in damar. In frogs the canaliculi are very regular and the posterior lamellæ easy to strip off.

No. 175.—Very good preparations of *fixed corneal cells* are obtained by the method of *Drasch*. The objects are not to be taken from the animal recently killed, but 12 to 24 hours after death, during which time the cadaver must be kept in a cool place. Small pieces of the cornea are to be cut out, about 6 mm. long, placed in 5 c.c. of 1 per cent. gold-chloride solution plus 5 c.c. of distilled water and stood in the dark for 1 hour. During this time stir the fluid often with a glass rod; then with glass rods transfer the pieces to 30 c.c. of distilled water, in which they should remain (in the dark) 8 to 16 hours. They are then to be transferred to 25 c.c. of distilled water plus 5 c.c. of formic acid and exposed to daylight. When the reduction is completed (p. 35) the dark-violet pieces are to be hardened in gradually strengthened alcohol, and in about 6 days thin sections, parallel to the surface, can be cut and mounted in damar (Fig. 224).

No. 176.—*Nerves and Blood-vessels of the Fresh Cornea.*—Select the eye of an ox and cut out the cornea and the portion extending from the limbus to the attachment of the ocular muscles; remove, with scalpel and forceps, the ciliary body, iris, and lens, cut out a quadrant of the cornea, place it with the epithelial side up on a slide, and apply a cover-glass; a drop of the vitreous humor may be added. The very thick preparation must be examined with a low power. At the scleral margin the loops formed by the blood-vessels as they bend back can be seen when the surface of the cornea is in focus; the most of them still contain blood-corpuscles. Medullated nerve-fibers are also found here, as well as in the deeper strata; they are arranged in bundles and within the cornea can only be traced for a short distance. The elongated pigment-streaks found in the eye of the ox have no relation to the nerves.

This method is not serviceable for the exhibition of the finer distribution of the nerves.

No. 177.—*Nerves of the Cornea.*—*a. Gold Method.*—Cut out the cornea 12 to 24 hours after death, remove the ciliary body and iris, and treat it according to the method given in No. 175. When the hardening is completed cut horizontal sections, which contain the epithelium and the uppermost strata of the cornea, and vertical sections through the thickness of the cornea. Mount in damar (Fig. 239).

b. Methylene Blue Staining.—Kill a rabbit; remove the entire eye-ball; free it from the attached remnants of ocular muscles and connective-tissue; place it in a watch-glass and with a sharp scalpel make a deep incision through all the coats of the eye at the equator. The vitreous humor thus escapes into the watch-glass; then with scissors separate, at the incision, the entire cornea, place it on a slide with the concave surface upward, and scrape off with the handle of the scalpel the ciliary body, iris, and lens, which is easily done; transfer the cornea thus cleansed to a second watch-glass containing 3 to 10 drops of the vitreous humor and 3 to 4 drops of a $\frac{1}{15}$ per cent. methylene blue solution. The fluid must cover the concave surface of the cornea.

The time required for staining cannot be given with certainty; it is therefore advisable after several hours to place the cornea with the convex surface up on a clean slide and, without a cover-glass, to examine it with the low power; if it is not sufficiently stained return it to the watch-glass and examine it again in about 10 minutes.

So soon as the nerves can be distinctly seen the cornea is to be transferred for from 18 to 20 hours to 20 c.c. of ammonia; then cut out a quadrant and mount it in dilute glycerine, to which a drop of ammonia has been added; after being kept for 24 hours in the dark the preparation will be sufficiently transparent and can be investigated with the high power.

No. 178.—*Lens-fibers.*—Cut the eye-ball open back of the equator; remove the vitreous body and lens; thus the pigment covering the ciliary processes remains attached to the margin of the lens. Loosen the lens from the vitreous body and place it in 50 c.c. of Ranvier's alcohol (p. 19). In about 2 hours thrust needles into the anterior and posterior surfaces of the lens and strip the capsule up from a small area; this is easily done; if lens-fibers are attached to the capsule it does not matter. On pricking the lens a turbid white fluid escapes; shake the alcohol and let the lens remain in it 10 to 40 hours. At the expiration of this time the lens can be easily separated into shell-like pieces. Tease a small strip of one of these pieces in a small drop of salt solution on a slide (p. 25). Apply a cover-glass, taking care to avoid pressure; if it is desired to preserve the fibers, stain with picocarmine (staining usually occurs in a few minutes), and mount in dilute acidulated glycerine (Fig. 236, *A*).

No. 179.—*Lens-fibers in Transverse Section.*—Place a lens in 50 c.c. of 0.05 per cent. chromic acid. A cloth or a little cotton must be placed on the bottom of the bottle or the lens will adhere to the glass and burst. This may also be prevented by frequently shaking the bottle. In 24 to 48 hours break up the lens into shell-like pieces with a needle, transfer them after 10 to 15 hours to 30 c.c. of 70 per cent. alcohol, which is to be replaced on the following day by an equal quantity of 90 per cent. alcohol. With the scissors cut the pieces through in the region of the equator, and so embed them in liver that the first sections shall pass through the zone lying next to the equator. If the section, which need not be very thin, has passed through the fibers transversely they will appear as sharply-defined hexagons; if, on the contrary, the section is oblique, the single fibers will appear to be separated from one another by irregular zigzag lines; they may even be cut partially lengthwise. The sections are to be transferred directly from the blade to the slide and mounted in dilute glycerine (Fig. 236, *B*).

No. 180.—*The Lens Capsule and the Lens Epithelium.*—Place the eye-ball, free from muscle and fat, in 100 to 200 c.c. of Müller's fluid. Treat it further as follows:—

a. Surface View of the Lens Capsule and Epithelium.—After 2 to 3 days cut the eye open, remove the lens, and with forceps strip off a piece of the anterior lens capsule; place it for about 5 minutes in a watch-glass with distilled water, which is to be changed once, and then stain it in Böhmer's hematoxylin; mount in damar. The capsule appears a homogeneous light blue; the nuclei and the contour of the epithelial cells are very sharp (Fig. 237, C). If it is desired to obtain the lens capsule alone strip off a portion of the posterior lens capsule.

b. Sections of the Capsule and Epithelium.—Let the eye-ball remain in Müller's fluid for 2 weeks; remove the lens, wash it for 1 hour in running water and harden it in 50 c.c. of gradually strengthened alcohol (p. 29); cut meridional sections through the anterior surface and the equator of the lens; stain them with Böhmer's hematoxylin (p. 31) and mount in damar (Fig. 237, D).

No. 181.—*The Blood-vessels of the Eye.*—For this purpose surface preparations are especially suitable. Open a fresh eye at the equator. The course of the central artery of the retina is macroscopically perceptible. For the exhibition of the blood-vessels of the choroid place an eyeball completely freed from attached muscle and fat on a small glass funnel which has been thrust into a low glass bottle, and, with scissors and forceps, begin at the equator and carefully dissect off the sclera. With a little practice the entire sclera can be removed beyond the ora serrata up to the optic entrance without injury to the choroid; care must be taken not to tear it. (Beginners should be content to remove only one quadrant of the sclera.) All the firmer points of attachment between the sclera and choroid (the *venæ vorticosæ*) must be cut through. Then by careful brushing with a sable pencil moistened in water remove the attached portions of the lamina suprachoroidea from the choroid; by this manipulation the course of the larger blood-vessels is brought to view. Thus far the investigation may be pursued on the uninjected eye (compare with No. 170, a). For the study of the blood-vessels of the ciliary body and the iris it is necessary to use an injected eye, divided anterior to the equator, fixed in Müller's fluid and hardened in alcohol. The iris and ciliary body may be easily stripped from the sclera; remove the lens and mount in damar. Examine first with the low power.

No. 182.—Place the *upper eyelid* of a child in 100 c.c. of 0.5 per cent. chromic acid, 1 to 3 days, wash it 2 hours in running water, and harden in 50 c.c. of gradually strengthened alcohol. For a general view cut thick (Fig. 240), for the finer details thin sections (Fig. 22, C). Staining with Böhmer's hematoxylin is at first difficult, but more readily accomplished after the object has lain in alcohol several months (compare p. 31, remark*). Mount in damar.

No. 183.—*The Lacrymal Glands.*—The lower *tear-gland* in man can be easily removed, without visible external injury, from the fornix of the conjunctiva. In the rabbit this gland is very small and when fresh resembles pale muscle tissue. It must not be confused with Harder's gland lying in the median angle of the eye. Treat like No. 112. Small pieces 1 mm. square can be used. The excretory duct and tubules may be easily seen; difficult, on the other hand, it is to see the intercalated tubules, whose epithelium differs greatly in height and occasionally is so low that care must be taken not to confuse them with blood-vessels.

XVIII. THE ORGAN OF HEARING.

A fundamental condition is an exact knowledge of the macroscopic anatomy of the labyrinth. The difficulties, the failures, depend in the main on inaccurate knowledge of the bony labyrinth. As a preliminary all parts lying lateral to the promontory (os tympanicum and ossicles of the ear) must be removed, so that this is distinctly visible.

No. 184.—*Otoliths*.—Chisel out the promontory, beginning at the upper margin of the fenestra stapedii, to the lower margin of the fenestra rotunda. Then, especially if the bone has been placed in water, the white spots (maculæ) in the sacculus and utriculus can be detected. With delicate forceps lift out the sacculus and spread out a small piece in diluted glycerine on a slide. The otoliths are present in large numbers, but are very small, so that their shape can only be distinctly seen with the high power (240 diameters). The glycerine must not be too thick, or it will render the otoliths completely invisible (Fig. 242).

In taking out the sacculæ portions of the semicircular canals not infrequently may be removed; stain these with picrocarmine and mount them in dilute glycerine. Only the epithelium, and here and there in optical section the delicate glassy membrane, can be seen. The connective tissue is scanty.

No. 185.—*The Cochlea*.—The base of the cochlea lies in the bottom of the internal auditory meatus, the apex is directed toward the Eustachian tube, and therefore the axis of the cochlea is horizontal and transverse to the long axis of the petrous bone.

Open the free portion of the cochlea, that is, remove the promontory close to the fenestra rotunda, open the apex of the cochlea and, having removed the superfluous osseous mass as far as practicable, place the preparation in 20 c.c. of 0.5 per cent. osmic acid (5 c.c. of 2 per cent. osmic acid to 15 c.c. of distilled water). In 12 to 20 hours wash the preparation for about 1 hour, and then place it in 200 c.c. of Müller's fluid. In 3 to 20 days (or later) open up the cochlea and examine it in water. The osseous spiral lamina can be seen as a delicate lamella, the membranous spiral lamina as a delicate membrane, attached to the axis of the cochlea; with fine forceps break off pieces of the osseous spiral lamina; do not lift them with the forceps, but carefully with needle and section-lifter remove them from the fluid and transfer them to a drop of dilute glycerine on a slide. It is advisable to break off the axial portion of the spiral lamina on the slide with needles, because the relatively thick osseous process renders it difficult to apply a cover-glass. The vestibular surface must be directed upward; it may be recognized by the auditory teeth, which are visible when the upper surface is in focus (Fig. 244), while the other portions are not distinct until the tube is depressed and the lower planes are focused. With the low power only the interstices of the auditory teeth are at first visible, as dark lines (Fig. 246); the papillæ likewise cannot be seen immediately, even with the high power, but become distinct after the second or third day. The chief difficulty lies not in the finishing, but in the proper examination of the object; the picture alters with the slightest change in focus. In Fig. 247, *B*, the membranous spiral lamina is drawn schematically, as seen with the upper surface in focus, and, therefore, only the free surface of the structure, drawn as seen from the side in *A*, is visible. It is clear that in depressing the tube the head-plates of the pillar-cells are no longer visible, but their bodies (as circles in optical section); the reticular membrane,

likewise, disappears, and can only be seen when the tube is elevated. The preparation may be stained with picrocarmine and preserved in dilute glycerine. The foregoing directions are intended to apply to the human ear and that of the cat. The labyrinths of children are to be recommended.

No. 186.—*Sections of the Bony and Membranous Cochlea*.—Remove the cochlea of a child from the labyrinth. The compact osseous substance of the cochlea is surrounded by spongy bone so soft that the latter may be removed with a stout penknife. With a chisel make small openings in the cochlea at two or three places, about 1 mm. square, in order to facilitate the penetration of the fixation fluid; then place it in 15 c.c. of distilled water plus 5 c.c. of 2 per cent. osmic acid. After 24 hours remove the object, wash it for a quarter of an hour in running water, and harden it in about 60 c.c. of gradually strengthened alcohol. When the hardening is completed, decalcify the cochlea in the following mixture: 1 c.c. of a 1 per cent. aqueous solution of palladium chloride, 10 c.c. of hydrochloric acid, and 100 c.c. of distilled water. Place the cochlea in 100 c.c. of this mixture, which must be changed often. When the decalcification is completed, the object should be hardened again, embedded in liver, and sectioned. The sections must be made in the long axis of the cochlea. Stain them with picrocarmine; mount in damar. It is not difficult to obtain preparations furnishing a good general view; the vestibular membrane is usually torn, so that the ductus cochlearis and scala vestibuli appear as a common space (Fig. 243). The organ of Corti leaves most to be desired; only very thin sections which pass through the organ vertically furnish intelligible pictures; usually a section contains several inner and outer pillar-cells, also fragments of them; the cells of Hensen appear pale and swollen (Fig. 249); orientation presents many difficulties to the beginner.

Among animals, the cochlea of the guinea-pig and of the bat are to be recommended; it is not embedded in spongy bone and does not need to be chiselled out and punctured, but can be placed at once in the fixing fluid.

No. 187.—*The Nerves of the Macula, Crista, and Cochlea*.—For this purpose the ear of the newborn mouse is recommended, treated according to the method given on p. 35. The base of the cranium, after removal of the vertex, the brain, and lower jaw, is to be placed for from 3 to 4 days in the osmio-bichromate mixture and for 2 days in the silver solution. As a rule it is necessary to employ the double method (p. 36). Cut horizontal and frontal sections through the cranium, without decalcifying it. The former are the more readily made.

No. 188.—*The Eustachian Tube*.—To obtain transverse sections (including cartilage and mucosa) the oblique direction of the tube downward, forward, and inward must be ascertained. Cut out the pharyngeal division of the tube together with the surrounding muscles and fix it in 200 to 300 c.c. of Müller's fluid (p. 27). In 3 to 6 weeks wash it in running water and harden it in 100 c.c. of gradually strengthened alcohol (p. 29). The sections may be stained in Böhmer's hematoxylin (p. 31) and mounted in damar (p. 38). For a general view, examine with the low power.

No. 189.—*The Ceruminous Glands*.—Cut out the ear with the cartilaginous auditory passage close to the bony auditory passage. From the cartilaginous portion cut a piece 1 cm. square and place it in 30 c.c. of absolute alcohol. The tissue may be sectioned on the following day. If it is desired to see the coil and the excretory duct the sections must be tolerably thick (—0.5 mm.). Nuclear staining with Böhmer's hematoxylin (p. 32) may be employed (Fig. 250). Examine thin unstained sections in diluted glycerine: in

these the fat-globules and pigment-granules can be seen. The organs of newborn children are especially suitable for this purpose. In adults the tubules are widely dilated and do not furnish good general views. On the other hand, the cuticular border of the gland-cells is distinct in the adult, which in the newborn I miss (compare with Fig. 251).

XIX. THE MUCOUS MEMBRANE OF THE NOSE.

No. 190.—*Olfactory Cells*.—Saw open the head of a rabbit in the median line. The olfactory mucosa is easily recognized by its brown color. With fine scissors cut out a small piece, about 5 mm. long, of the mucosa, together with the corresponding portion of the turbinal bone, and place it in 20 c.c. of Ranvier's alcohol (p. 19). In 5 to 7 hours transfer the same to 5 c.c. of picrocarmine and on the following day to 10 c.c. of distilled water. In about 10 minutes remove the piece and lightly strike it against a slide on which a drop of diluted glycerine has been placed; stirring with the needle is to be avoided. Carefully apply a cover-glass. In addition to many fragments of cells many well-preserved sustentacular elements may be obtained. Very frequently the delicate central process of the olfactory cells is wanting (Fig. 253).

No. 191.—*The Mucous Membrane of the Respiratory Region*.—Cut out a small piece, about 5 to 10 mm. long, from the lower half of the nasal septum; strip off the mucosa and fix and harden it in about 20 c.c. of absolute alcohol (p. 27). Use the nasal mucous membrane of the rabbit's head (No. 190) for thin sections; embed the pieces in liver (p. 31), and stain sections with Böhmer's hematoxylin; mount in damar. For general views the mucous membrane of human cadavers answers, which is to be treated in the same manner; thick, unstained sections are to be mounted in diluted glycerine (Fig. 252).

No. 192.—*The Mucous Membrane of the Olfactory Region*.—Remove pieces 3 to 6 mm. long of the brown mucosa from the upper portion of the nasal septum of a rabbit (No. 190), and place them for 3 hours in 20 c.c. of Ranvier's alcohol, which loosens somewhat the elements of the olfactory epithelium. Transfer the pieces carefully to 3 c.c. of 2 per cent. osmium solution plus 3 c.c. of distilled water, and place the whole for from 15 to 24 hours in the dark. At the expiration of this time the pieces are to be placed for a half hour in 20 c.c. of distilled water and then hardened in 30 c.c. of gradually strengthened alcohol. The hardened pieces are to be embedded in liver and sectioned. Stain the sections 20 to 30 seconds in Böhmer's hematoxylin; mount them in damar.

In order to obtain good views of the *glands* make thick sections transverse to the course of the *nerve-fibers* (Fig. 255). For the exhibition of the *nerve-fibers* and the epithelium thin sections parallel to the course of the fibers are suitable (Fig. 256).

No. 193.—*The nerve-processes of the olfactory cells* may be obtained in preparations made according to No. 178. In these the duct-system of the olfactory glands is often blackened.

No. 194.—*For orientation with regard to the number and position of the taste-buds* proceed according to the method in No. 96. Suitable objects are the circumvallate papillæ of any animal and the papillæ foliatae of the rabbit. The latter consist of elevated groups of parallel folds of the mucosa, found one on either edge of the root of the tongue. In moderately thin sections vertical to

the long axis of the folds, examined with the low power, the taste-buds may be recognized as clear spots.

No. 195.—*The Structure of the Taste-buds*.—Dissect off with scissors a papilla foliata of a rabbit, with as little as possible of the subjacent muscle substance. Pin the piece with spines on a cork-stopper, the muscle side toward the cork, and expose it for 1 hour to the vapor of osmic acid (see further p. 28, 6). Thin sections of the hardened preparation embedded in liver are to be stained 30 seconds in Böhmer's hematoxylin and mounted in damar (Fig. 258).

No. 196.—*Exhibition of the Nerves*.—Cut out with scissors a circumvallate papilla (without the wall), and place it for 10 minutes in the filtered juice of a lemon; then transfer it to 5 c.c. of a 1 per cent. gold-chloride solution and place the whole for 1 hour in the dark. Lift the papilla with wooden rods from the gold-chloride solution into a watch-glass with distilled water and wash it by moving it to and fro. Transfer it to 20 c.c. of distilled water to which 3 drops of acetic acid have been added. In this expose the papilla to daylight until the reduction is completed, which usually requires 3 days. Harden the papilla, in the dark, in 30 c.c. of gradually strengthened alcohol. Embed the object and make the thinnest possible sections. Mount in damar. The nerve-fibers are dark-red to black, the gustatory cells are also dark (compare with Fig. 259).

The papillæ foliatæ of the rabbit are not suitable for such preparations, but yield successful preparations by Golgi's method (p. 35). Place the papillæ for 3 days in the osmio-bichromate mixture, for 2 days in the silver solution. The double method is to be recommended. The intergemmal fibers are more numerous and more readily blackened than the intragemmal fibers, which are exceedingly delicate. Frequently single cortical and gustatory cells become blackened.

APPENDIX.

MICROTOME TECHNIQUE.

THE MICROTOME.

The most useful microtomes are constructed according to two different principles.

The principle of the one kind consists therein, that the object to be sectioned is elevated by the shifting of the object-holder up an inclined plane.

In the other form, the object is elevated in a vertical direction by a micrometer-screw.

Both kinds are excellent instruments.* All parts of the microtome should be kept as clean as possible. It should be protected from dust, when not in use, by covering it with a light wooden case. The slideway in which the knife moves must be kept scrupulously clean. It should be cleansed occasionally with a cloth moistened in benzine and should then be freely lubricated with vaseline, so that the sliding-block will pass evenly throughout the entire slideway at the lightest touch. Especial care must be bestowed upon the knife. Only with a very sharp knife can very thin sections be made or ribbon cutting be done. A really sharp knife should pass easily through a thin hair held at one end between the fingers.

EMBEDDING.

PARAFFIN METHOD.

The following materials and apparatus are required:—

1. *Paraffin*: two kinds, a soft (melting point 45° Celsius) and a hard (melting point 52° Celsius). Of this prepare a mixture which melts at 50° Celsius. On the proper proportions of the two sorts of paraffin in the mixture much depends. Many a failure is due to an unsatisfactory mixture. The precise proportions cannot be given because the consistence of the paraffin depends in a great measure on the outer temperature. Then, too, hard objects, as well as the cutting of very thin sections, require a harder mixture than usual. For winter, at a room-temperature of 20° Celsius, a mixture of 30 grams of soft and 25 grams of hard paraffin † answers for most purposes.

* The workmanship of the sliding microtomes of Thoma, made by Jung in Heidelberg, is exquisite, as I know from my own experience. The size No. IV is especially to be recommended. For several years I have used the microtome of Schanze in Leipzig, Model B, No. 9, the construction of which leaves nothing further to be desired. The microtomes constructed on the same principle, by G. Mihe in Hildesheim, are also to be highly recommended; and very good are those of A. Becker in Göttingen. [A very satisfactory sliding microtome is made by the Bausch and Lomb Optical Company of Rochester, N. Y.]

† To be obtained of Dr. Gröbler, Leipzig.

2. *Chloroform* : 20 c.c.

3. *Paraffin-chloroform* : a saturated solution (5 grams of the paraffin mixture and 25 c.c. of chloroform). This solution is liquid at room-temperature.

4. *An embedding oven* of block-tin with double walls, between which is a space to be filled with water. * A small gas-burner is to be placed beneath the oven. On top there are two openings ; the one leads into the space between the walls, and into this a Reichert thermo-regulator † is to be inserted ; the second opening leads into the air-space or oven, and into this a thermometer is to be inserted. The front wall consists of a glass plate which slides up and down in grooves. The interior of the oven is divided into three compartments by means of two adjustable shelves. The oven should be 25 cm. long, 23 cm. high, and 16 cm. deep. The embedding oven, with its accessories, is indispensable if much embedding in paraffin is to be done ; however, the paraffin may be melted on a water-bath and kept liquid with a small spirit-flame.

5. *An Embedding Frame*.—This consists of two adjustable bent metal frames, placed together thus

Instead of this frame little paper trays made of stiff paper or cardboard can be used. The objects to be embedded must be absolutely free from water, and to this end should have lain 3 days in absolute alcohol, which has been changed several times ; they are then to be transferred to a bottle containing 20 c.c. of chloroform, in which they should remain until the following day. From this the objects should be carried to the solution of paraffin in chloroform and, in from 2 to 8 hours, according to their size, transferred to a capsule containing melted (but not too hot) paraffin. In about a half hour the objects are to be transferred to a second capsule with melted paraffin. ‡ where, according to their size, they are to remain from 1 to 5 hours. § The paraffin should not be heated more than 2 to 3 degrees above its melting point ; for the mixture advised the air in the oven should have a temperature of 50° Celsius. When the objects have been in the paraffin bath the required length of time, place a slide in a broad dish and on this the embedding frame, into which the paraffin and object are now to be poured. Then, while the paraffin is still fluid, with a heated needle place the object in the desired position ; so soon as this is done carefully pour cold water into the dish until it reaches the upper margin of the frame ; the paraffin will begin at once to harden, whereupon more water may be added until the entire frame is submerged. By this manipulation the paraffin hardens into a homogeneous mass, whereas otherwise it is apt to crystallize and is then difficult to cut and also has an injurious influence on the structure of the embedded tissues. In about 10 minutes the metal frames may be removed ; the paraffin block should be allowed to remain in the water on the slide until it is completely hard.

The embedded object may be sectioned in a half hour. In case it is to be used later mark it with a needle. In the paraffin the object can be kept for an indefinite period.

* Made by R. Jung, Heidelberg.

† To be obtained of Reichert, Vienna.

‡ If the paraffin has been melted on a water-bath, place the flame at such a distance that the surface of the paraffin remains covered by a thin film.

§ This is sufficient for all cases ; for small objects from 1 to 2 hours will be enough.

CELLOIDIN METHOD.

Two solutions are required :—

a. A thin solution of about 30 grams of celloidin cut into cubes are to be dissolved in 60 c.c. of a mixture of equal parts of absolute alcohol and ether.

b. A somewhat thicker solution of 30 grams of celloidin dissolved in 40 c.c. of a mixture of equal parts of absolute alcohol and ether. This solution has the consistence of a thick syrup.

Both solutions should be kept in wide-necked bottles. If they become too thick they may be thinned by the addition of some of the alcohol-ether mixture. After a time the solutions become turbid and milky ; it is better then to let them dry completely and to redissolve the pieces in the alcohol-ether mixture.

The tissues to be embedded must be completely free from water and must have lain 1 to 2 days in absolute alcohol, which has been changed several times. From this the objects should be transferred to the thin, and on the following day to the thick, celloidin solution. In the latter, the objects may remain for an indefinite length of time. Usually they are sufficiently permeated after 24 hours, but large objects enclosing many spaces must remain in the thick solution about 8 days. The object should then be quickly placed on a cork-stopper and some celloidin poured over it. In doing this care must be taken not to press the object against the cork, lest it become detached. There should be a stratum of celloidin 1 to 2 mm. thick between the cork and the object.* Now the whole is to be placed under a bell-glass to dry slowly ; the bell-glass should not be air-tight, and to avoid this should be supported on one side on a needle or something similar. Delicate objects dry in a half hour, larger objects in 4 hours ; they are then to be placed in a glass jar with 30 c.c. of 80 per cent. alcohol. In order that the objects may be submerged, glue the under surface of the cork-stopper by means of celloidin to the inner surface of the lid of the jar. On the following day the alcohol should be replaced by 70 per cent. alcohol, in which the tissue may remain an indefinite length of time.

In order to cut thin sections the celloidin must be hardened ; for this purpose transfer the objects embedded in celloidin from the 80 per cent. alcohol for 2 days or longer into an alcohol-glycerine mixture (80 per cent. alcohol one part, pure concentrated glycerine 6 to 10 parts). The larger the proportion of glycerine to alcohol, the harder the celloidin becomes. This mixture may be differently prepared ; an extreme limit is 1 part of alcohol to 30 parts of glycerine. Still greater difference in the proportions produces strong curling of the sections. In order to prevent the yielding of the elastic celloidin block, dry it carefully with filter-paper on removing it from the alcohol-glycerine mixture ; make a pair of lateral incisions and dip it into liquid paraffin ; such blocks cannot be preserved dry—they must be returned to the alcohol-glycerine mixture.

Preparations fixed by Golgi's method require special treatment, since the absolute alcohol has an injurious influence if the object remains in it beyond 1 hour. When the tissue is taken from the silver solution it is to be placed in 30 c.c. of 96 per cent. alcohol, 15 to 20 minutes, then hardened in absolute alcohol for 15 minutes, and then placed in the thin celloidin solution for 5 minutes. Meanwhile, in the previously smoothed lateral surface of a broad piece of elder-

* This stratum must not be thicker ; even well-hardened celloidin is elastic, and a thick layer would cause the object to give in sectioning.

pith, make an excavation just large enough to take in the whole preparation ; insert it, cover it with celloidin solution, and then fit a second piece of elder-pith on the first, pour on more celloidin, and place the whole for 5 minutes under a bell-glass to dry ; then transfer it to 80 per cent. alcohol for 5 minutes, and cut sections with a knife flooded with 80 per cent. alcohol. The microtome is altogether unnecessary ; satisfactory sections can easily be cut free-hand. If the microtome be used, the thickness of the sections should vary from 40 to 120 μ . The elder-pith should be trimmed off so that only a small border (1 mm.) surrounds the celloidin.

SECTIONING.

Paraffin Objects with the Knife Placed Obliquely.—The paraffin block containing the tissue is to be secured in a hollow cylinder coated with hard paraffin (in the Thoma microtome) or (in the microtome of Schanze) to a little plate adjoining the clamp. With the latter the plate is simply warmed and the paraffin block glued to it by pressure. In the case of the cylinder, warm it and also the base of the paraffin block ; press the latter lightly into the cylinder and by means of a heated needle inserted between them establish a firm union. In order to cool the paraffin quickly place the cylinder or the plate for 5 minutes in cold water. The projecting portion of the paraffin block containing the object should then be trimmed to a four-sided column, the base of which is a right-angled parallelogram.

The column must not be taller than 1 cm., and the object should be covered by a layer of paraffin not over 1 to 2 mm. thick. The cylinder (or the plate) with the object should now be placed in the microtome. Sections are to be cut with the blade of the knife dry. The position of the knife depends on the nature of the object.

Sectioning with the Knife Placed Obliquely.—If the object is large and of unequal resistance the knife should be so clamped that it forms a very acute angle with the long axis of the microtome. The paraffin block should stand so that the knife strikes it first on one corner. The knife should be moved slowly in the slideway and pressure upon it should be carefully avoided.

Sectioning with the Knife Placed Transversely.—Screw the knife down perpendicular to the long axis of the microtome, turn the paraffin block so that the blade will strike it first on a flat surface. The knife should be moved rapidly with a planing movement and then the sections will adhere to one another at their edges and form long ribbons. When the paraffin is of the right consistence the first section lies smooth on the blade and is shoved by the second section in the direction of the back of the blade. If however the first sections show an inclination to curl and fall over the edge, they must then be carefully held with a delicate sable brush and led back to the right direction. Ribbon cutting is most successful when the sections have a thickness of 0.01 of a mm. ; thicker sections curl easily and do not readily adhere to one another at their edges.

Obstacles in Sectioning and their Remedy.—Every one who has worked with paraffin is probably able to explain many an unsuccessful attempt.

1. The knife glides over the object and cuts a partial section or none. The reason for this may lie in the microtome ; the slideway may not be clean ; examine the vertical portion of the slideway. Or the knife is not sharp enough, or the under surface has paraffin attached to it ; in the latter case remove the knife and with a cloth wetted with turpentine carefully cleanse it. Knives with thin backs buckle when the distal end of the blade is used ; thus it happens that when the knife is obliquely placed the blade strikes the

tissue at first and glides over the rest without cutting it. In microtomes of earlier construction the cause of this often lies in the unsatisfactory manner in which the block of paraffin is secured.

Secondly, the trouble may be found in the object; it may be too hard, or of very unequal resistance, or poorly embedded; in the latter case there are two possibilities. Either the preparation was not thoroughly dehydrated, in which case it exhibits opaque spots, or it still contains chloroform; in this case it is soft, and light pressure with a needle on the surface leaves a mark or even presses out fluid. In both cases the procedure of embedding must be repeated, reversing the series of processes to the absolute alcohol (in the latter case to the paraffin bath).

Finally, the consistence of the paraffin may be at fault.

2. The sections curl. This can be prevented by holding a small sable brush or bent needle lightly against the curling sections.* The cause of this curling lies in the hardness of the paraffin, which is also responsible for—

3. The sections break. The serviceableness of the paraffin depends in a high degree on the outer temperature. If the paraffin is too hard do not endeavor to reduce its consistence by the admixture of soft paraffin,—this is the last resource,—but employ simpler measures. Cut the sections near a stove or near a lamp; often slight warming of the knife is sufficient. Even very good paraffin crumbles when cut with a cold knife.

4. The sections fold and become pressed together. As a result of this the sectioned objects acquire a false form. The reason for this lies in a too soft paraffin. This difficulty may be overcome by placing the block frequently in cold water or by cutting the sections in a cold room (in summer, in the morning hours).

Celloidin Objects.—The embedded object is to be trimmed so that it is surrounded by a stratum of celloidin only 1 to 2 mm. thick; clamp the knife obliquely, so that it makes a very acute angle with the long axis of the microtome. The knife must be moistened with 70 per cent. alcohol, by means of a sable brush; this must be repeated after every second or third section. The sections should be removed with a sable brush and transferred to a dish containing 70 per cent. alcohol. Very thin sections (less than 0.02 mm.) cannot be cut unless the celloidin has been hardened.

PRESERVATION OF THE SECTIONS.

Paraffin Objects.—If the sections are not very thin and are not in ribbons, they may be placed in a capsule with 5 c.c. of turpentine, and when the paraffin is dissolved transferred to a second capsule with turpentine. From this the sections, if the tissue has been stained in bulk, are brought on to a slide and mounted according to the directions given on p. 38. If the sections are unstained, transfer them from turpentine to 5 c.c. of absolute alcohol, which is to be changed in 5 minutes. In another 2 minutes the sections may be stained. In the case of serial sections and very thin sections, it is necessary to fasten the dry sections on the slide. The slide must be absolutely clean; wash it with alcohol and dry it with a clean, *not oily*, cloth, or place it for a half hour in cold soap-suds. On the well-dried slide arrange the sections (or portion of the "ribbon"), and at the edge of the same place a drop of distilled

* A "section-smoother" for microtomes in which the object is elevated vertically is made by Kleinert of Breslau. See further Born, "Zeitschr. f. wissensch. Mikroskopie," Bd. x, p. 157.

water by means of a sable brush. Another section (or portion of the ribbon) is now to be placed on the slide, another drop of water added, and so on until the slide is covered. It does not matter if the sections float. Pass the slide through a spirit-flame or place it 1 to 3 minutes in the oven; * on being slightly warmed, the sections spread out flat and smooth. Then arrange them with a needle, and by slightly inclining the slide let the water flow off, or absorb it with a strip of filter-paper and, protected from dust, let the whole dry. On the following day pour turpentine over the slide and, if the sections are already stained, mount them in damar. In case the sections are not stained the turpentine is to be wiped off and the slide placed in absolute alcohol.† After 5 minutes take the slide from the alcohol, which is to be quickly wiped off around the sections, and either placed in the stain or covered with a drop of the solution. Then slowly transfer the slide to a dish with distilled water and preserve it in dilute glycerine (p. 37), or with the customary preliminary treatment with absolute alcohol and oil of bergamot (p. 38), mount it in damar.

Celloidin Objects.—Place the sections in a dish containing 20 c.c. of 90 per cent. alcohol. If the tissue has not been previously stained in bulk, staining in bulk is to be preferred, the sections may be subsequently stained; but aniline colors cannot be used, as these also stain the celloidin; even hematoxylin imparts a light blue tint to the celloidin. The sections must not be placed in absolute alcohol, since this dissolves the celloidin; they are to be taken from the 90 per cent. alcohol and placed in chemically pure amyl alcohol and then transferred to xylol; when the clearing is completed (p. 38) mount them in xylol-balsam.

Serial sections of celloidin objects are only used for special purposes, for example, for the central nervous system. See the articles by Wiegert in the "Zeitschrift für wissenschaftliche Mikroskopie," Bd. ii., p. 490, Bd. iii., p. 480, Bd. iv., p. 209. The negative varnish recommended in the article is to be obtained of Dr. Grübler.

* The paraffin must not be allowed to melt; the resulting mixture of melted paraffin and water is not soluble in turpentine.

† The turpentine, also the alcohol, must be quickly wiped off, because the sections are rendered useless if they are allowed to become dry. Care must also be exercised in placing the staining fluid on the sections, which it should completely cover. Loosening of the sections occurs when there is not enough water between the section and the slide—the water must be evenly diffused between the two. The sections may also be fastened to the cover-glass, but this method necessitates the use of larger quantities of the staining solution, alcohol, and other reagents.

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