NOTES

— ON —

HISTOLOGICAL METHODS

INCLUDING A BRIEF CONSIDERATION OF THE METHODS OF

PATHOLOGICAL AND VEGETABLE HISTOLOGY,

AND THE APPLICATION OF THE MICROSCOPE TO JURISPRUDENCE.

For the use of Laboratory Students in the Anatomical Department of the CORNELL UNIVERSITY,

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Prefatory Note.

The object of histological methods is to assist the investigator in obtaining a complete knowledge of the tissues. Complete knowledge of any tissue comprises, in the writer's opinion, an understanding of:

1. The gross anatomy.
2. The form, nature and relations of the structural elements.
3. The blood vessels.
4. The lymph-vessels.
5. The nerve-supply,—the relation of the terminal filaments of the nerves to the structural elements.
6. The histogenesis or development.
7. The function or physiology of the tissue.

At the present day, however, not a single tissue is known in all the detail indicated above.

The following pages are designed to supplement the notes printed in 1881, and to present improved methods, introduced since that time. A list of topics considered in the entire course is also given. The permanent preparations required of each student are thought to include those tissues, the knowledge of which is essential as a basis of histology; and the methods for their study, illustrate the principal methods of histological research—methods which, by combination or slight modification, will enable the student to obtain a good knowledge of all the tissues.

When not otherwise stated, the methods here given were taken from the books and periodicals named at the end; and after the methods of study of a tissue are given, references are made to the works in the list in which figures and a more or less complete account of its structure may be found. Finally, for the benefit of students specially interested in Botany, Medicine, and the Relations of Medicine to Law, three extra lectures are given on the methods of *vegetable* and *pathological histology*, and the application of the microscope to *jurisprudence*.

Special references to the books and periodicals in the alphabetical lists at the end are made by giving in brackets: 1st. If a book, the

initial letter of the author's name, or if more than one author has the same initial, the initial and one or more of the following letters in the name. For periodicals, the initial letters of the title are given. 2d. If the work consists of more than one volume, the volume is indicated by a Roman numeral, or with periodicals, the year instead of the volume is sometimes indicated by Arabic numerals in parentheses. 3d. The pages referred to are given in Arabic numbers. Finally, cross references to the various sections in these notes are given in parentheses.

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Topics.

1. **The Microscope and its Parts.**—Simple microscope—compound microscope—optical parts of a compound microscope—nomenclature of oculars—of objectives—dry, immersion and homogeneous objectives—working distance—mechanical parts. Choice of a simple, and of a compound microscope for laboratory use. Care of the mechanical, and of the optical parts. Care and training of the eyes in microscopic work. (Notes of '81. **C. Bi. Bau. R. Fi. H.** (J.r.m.s.) (Z.w.m.) **A.s.m. J.n-y.m.s**).

2. **Learning to use a microscope.**—The simple microscope. Putting oculars and objectives in position and removing them—focusing—with low and with high objectives. Putting an object under the microscope. Revolver or nose-piece. Field of the microscope. Lighting by direct or transmitted, central or axial and oblique light. Diaphragms. Demonstration of the function of the objective,—of the ocular—of the mirror. (Notes of '81. **Bi. C. Fi. H. R. St.**).

3. **Slides and Covers, Interpretation of Appearances.**—Slides and cover-glasses, cleaning and care (§ 1–4). Mounting an object, (§ 5.) Dust or cloudiness on the ocular, on the objective. Muscæ volitantes in the eye of the observer. Relative position of objects having plane outlines—having curved outlines—air bubbles and oil globules. Distinctness of outline depends on difference in color or refractive power. Optical section—*highly refractive—doubly contoured.* Currents—Pedesis or Brownian movement. Ranvier's demonstration of pedesis with polarized light (R. 175). (Notes of '81. **Bi. C. F.i.** R. Popular Scienee Monthly, Vol. VII, p. 177. Journal of Science, 1878, p. 167.)

4. Magnification, Micrometry and Drawing.—Magnification of a simple microscope—of a compound microscope. Obtained: (A.) Directly as for the simple microscope, (B.) With a camera lucida. Forms of camera lucidas and their action (Wollaston's, Abbe's Grunow's and Schrœder's). Notes of 81. Bi. C. R. (Z. w. m. I. 1–23.) J. r. m. s. (1883) p. 813, (A. m. m. J. III, 201.)

Micrometers and Micrometry—Optical combination and length of body—three ways of varying the magnification of a compound microscope.—(a) by dividing the size of the image by the magnification; (b) by using the camera lucida and stage-micrometer; (c) by dividing the real image as measured, by the ocular micrometer, by the ocular micrometer ratio. (Notes of '81.) Accuracy depends on (a) the perfection of the instruments, and (b) the skill of the observer. Notes of '81. Bi. C. Fi. R.).

Drawing.—Getting outlines with a camera lucida. Filling in details freehand. Getting image of a desired size. Drawing lines of a stage-micrometer near the drawing to indicate the magnification. (Bi. C. Fi. (Z. w. m. I. pp. 23.) (J. r. m. s. 1884, p. 697).

5. The Study of living tissues. (§ 7–20).
6. Isolation and preservation of the structural elements. (§ 21–32).
7. Hardening tissues. (§ 35–37).
8. Section cutting and mounting. (§ 34, 38–64).
9. Serial sections. (§ 60).
10. Fine injections. (§ 61–67).
11. Methods of pathological histology. (§ 68).
12. Methods of vegetable histology. (§ 69).
13. The microscope in Jurisprudence. (§ 70).
14. Reagents and their preparation. (§ 71).
15. Bibliography. (I–VIII).

PERMANENT PREPARATIONS.

The following permanent preparations are to be made, labelled, catalogued (§ 25), and submitted for examination and criticism the last week of the term. The cross references following each tissue, are to the sections in these notes where the methods of preparation are given, and references are made to books or periodicals in which may be found *figures* and *descriptions* of the tissue. Except when otherwise stated the tissues are from the *Cat.*

1. Blood of Necturus, (§ 21–23).
2. Blood of Man, (§ 24).
3. Ciliated epithelial cells, isolated, (§ 26).
4. Striated muscular fibers, isolated, (§ 27).
5. Muscular fiber-cells, isolated, (§ 28).
6. Cardiac muscular fibers, isolated, (§ 29).
7. Myelinic nerve-fibers, isolated, (§ 30).
8. Amyelinic nerve-fibers, isolated, (§ 31).
9. Areolar connective tissue, (§ 32).
10. Endothelium, (§ 33).
11. Section of cartilage, (§ 34).
12. Transection of a nerve, (ulnar, brachial, or sciatic), (§ 47).
13. Transection of myel, (cervical or lumbar enlargement), (§ 48).
14. Transection of a striated muscle, (occipito-scapularis,) (§ 49).
15. Transection of an artery, (§ 50).
16. Transection of trachea, (§ 51).
17. Section of submaxillary gland, (§ 52).
18. Section of stomach of Necturus, (§ 53).
19. Section of ileum, (§ 54).
20. Section of liver, (§ 55).
21. Section of ovary, (§ 56).
22. Section of human skin, (§ 57).
23. Section of injected kidney, (§ 63).
24. Section of injected small intestine, (§ 64).
25. Web of frog's foot with both blood and lymph vessels injected, (§ 67).

MICROSCOPIC SLIDES AND COVER-GLASSES.

(§ 1) **Slides.**—These are strips of clear, flat glass upon which microscopic specimens are mounted (§ 5). The size most commonly used in America is 1x3 inches. In this laboratory, slides 25x45 mm. are employed. Slides may be prepared very cheaply from strips of clear glass with a diamond or a steel glass-cutter.

(§ 2) **Cleaning Slides.**—New slides can usually be well cleaned by washing in soft water. Slides that have been used for mounting objects can be cleaned by soaking them in the cleaning mixture for glass (§ 72), and then washing in water. After the slides are well rinsed, take them singly and by opposite edges, and wipe dry with a soft clean cloth.

(§ 3) **Cover-glasses.**—These are circular or quadrangular pieces of glass used in covering microscopic objects. They should be very thin,—.10 to .18 mm. (1-250 to 1-150 in.) in thickness. Covers may be made very cheaply from the laminæ or scales of mica, but mica is too soft to be used for permanent preparations.

(§ 4) **Cleaning Cover-glasses.**—New cover-glasses should be put one by one into the cleaning mixture for glass (§ 72), and allowed to remain two days or longer. Cover-glasses that have become soiled in any way should be placed in the cleaning mixture for a week or more. Finally rinse the covers by a gentle stream of water till the color of the cleaning mixture is no more visible. In *wiping the cover-glasses*, grasp them singly and by opposite edges between the thumb and index of the left hand. Cover the thumb and index of the right hand with a soft, clean cloth, grasp the cover and rub its two surfaces. It is necessary to have the ball of the thumb and finger on exactly opposite sides in wiping the covers, or they will be broken. When a cover is well wiped, hold it up and look through it toward some dark object. It will be seen partly by transmitted and partly by reflected light. If it does not look clear, breathe on the faces and wipe them again.

Handle the cover-glasses and slides by their edges, or handle the cover-glasses with fine forceps. Do not touch the surface with the fingers.

As the covers are wiped, place them in pill-boxes, and assort them according to thickness. One can judge of the thickness of a cover-glass by looking at its edge. It is better, however, to measure the thickness of each cover as it is wiped, with Zeiss' Deckglass-Taster, and place those of like thickness in the same box (see § 25).

(§ 5) **Mounting microscopic objects.**—This is preparing them for study with the microscope. They are generally put upon slides (§ 1), and in some liquid called the *mounting medium*. To protect them from evaporation, dust and mechanical injury they are covered with cover-glasses (§ 3).

(§ 6) **Putting on the cover-glass.**—Grasp a clean cover-glass of the proper size near one edge with the fine forceps, and if necessary, dust on both sides with a quill duster. Without relaxing the grasp of the forceps, rest one edge of the cover on the slide near the object and slowly lower the other till the cover is nearly parallel with the slide, then remove the forceps and the cover will fall into position. If this method is followed, the cover-glass may be put exactly where it is wanted and there will also be avoided the displacement of the object and the inclusion of air-bubbles.

LIVING TISSUES.*

The student is advised to read all that is said upon each special subject before commencing work upon that subject.

(§ 7) **Normal conditions.**—For the best results in the study of living tissues it is necessary to maintain the normal temperature and, to surround the object with a normal fluid,—a fluid in which it naturally lives. For Protozoa and other aquatic animals, the water in which they are found is their normal fluid. For the blood-corpuscles of Necturus, frog and man, and for the ciliated epithelium from the frog, artificial serum made from egg-albumen, salt and water, answers very well (§ 73).

AMOEBOID MOVEMENT.

(§ 8) **Amoeba.**—This unicellular animal shows admirably the amoeboid movement,—a continual change of form due to the protrusion of one or more processes from any part of the circumference. Amoebæ are especially abundant in soft water regions. Their special habitat is the light, superficial ooze at the bottom of still waters. They are also usually abundant in the floculent matrials and slimy matter adherent to submerged stems and leaves, and in wet sphagnum moss. [*Leidy, 8–13, H. & M., 17*].

Mounting the amoebæ.—Grasp some of the floculent matrial and dead leaves at the bottom of the aquarium with the fine forceps and press them on the center of a clean slide several times. Cover (§ 6) the water and other material left on the slide by this process, and examine under the microscope, using a 1–5 objective. Every irregular translucent mass should be watched for a short time to see if it exhibits the amoeboid movement. It is not safe for a beginner to call any object an amoeba before it has exhibited the amoeboid movement. After once becoming familiar with amoeboid movement it is quite easy to detect moving amoeboid forms whether they are independent organisms like the amoeba or parts of higher animals like the white blood-corpuscles.

(§ 9) **White blood-corpuscles of Necturus.**—To obtain the white blood-corpuscles, pith a Necturus. To do this, remove the animal from the water; grasp it in a dry towel; wipe the dorsal part of the head dry; ventriduct the head, and press the thumb nail quite firmly

* Several free-hand drawings of an amoeba and of each kind of white blood-corpuscle should be made so that their various phases may be compared. Drawings of an infusorian and a ciliated epithelial cell should also be made.

against the dorsal side nearly opposite but slightly cephalad of the base of the first gill, to find the notch between the atlas and skull; pith the Necturus by forcing the tracer through the skin and muscles into this space, and thus severing the medulla. Then force the tracer into the cranial cavity and destroy the brain to remove the possibility of suffering.

(§ 10) **Mounting the corpuscles.**—After pithing it is well to moisten the place where the cut was made with the normal fluid. Usually a mixture of blood and lymph exudes from the wound. Moisten a cover-glass with normal fluid and touch it to the exuded fluid. Then place the cover, blood side down, upon the center of a slide which has been moistened with normal fluid and on which a short piece of hair is placed. The hair prevents the too great pressure of the cover upon the blood. The normal fluid is for convenience. If one does not possess it, he should breathe upon the cover and upon the slide. The moisture of the breath prevents the too rapid desiccation. If blood cannot be obtained where the animal was pithed, a gill may be wiped dry, then moistened with normal fluid and a slight cut made in it with scissors. Blood unmixed with lymph runs out and should be mounted as directed above, except that more normal fluid should be used to dilute it so that the red corpuscles shall not hide all the white ones. The best place to get white corpuscles, however, is either from the pericardial sac or the abdominal cavity. A dropping-tube is very convenient to get a drop of the liquid in these cavities after a small opening is made. Seal as in § 11. After the preparation is sealed put it under the microscope and examine with a 1–5 or higher objective and demonstrate the following: (A) The colored, oval, nucleated corpuscles; (B) Small white corpuscles nearly circular in outline and exhibiting slight or no amoeboid movement; (C) Large white corpuscles, some of them nearly as large or even larger than the red ones. These exhibit very marked amoeboid movement. The nucleus is in most cases very distinct and also undergoes apparently independent amoeboid changes [S. VII, 35].

(§ 11) **Sealing for blood.**—As it is sometimes necessary or desirable to study a fresh preparation for a considerable time—an hour or more—it is necessary to prevent evaporation. This may be done by painting a ring of castor oil around the cover-glass so that half of the ring shall be on the cover-glass and half on the slide. A ring of the melted paraffin sealing mixture is to be preferred, however (§ 75).

(§ 12) **White blood-corpuscles of a frog.**—To obtain the white corpuscles, proceed to pith as described for Necturus (§ 9). The notch between atlas and skull in the frog is nearly opposite the caudal margin of the membrana tympani. It is more easily found than in Nec-

turus. Mount and seal the blood from the pithing wound or from the pericardial or abdominal cavity, as described for Necturus (§ 9–11), and examine it in the same way. Demonstrate the presence of both red and white corpuscles and the marked amoeboid movement of the white ones; observe also the colored corpuscles [Quain II, 9, 23; (Q. j. m. s. (1871) 361); R. 148, 178].

(§ 13) **White blood-corpuscles of man.**—To obtain the white corpuscles of man, flame a needle (heat it red hot in an alcohol or gas flame), and after it is cool insert it obliquely a short distance into one of the fingers, then remove it and ligate the finger with a string or rubber band and also squeeze it. A drop of blood will exude. This should be mounted and sealed as described above (§ 9–11). The blood of man contains few white corpuscles as compared with the red ones,—1 to 350 or 500 [R. 211]. The average size of the white ones is greater than that of the red ones (red ones 7 to 8 μ, white ones when circular in outline 8 to 10 μ [R. 185, 210; Quain, II, 24, 29]). They undergo amoeboid changes as do those of the frog and Necturus. Marked amoeboid movements occur in the corpuscles of many persons after the blood is mounted for the microscope if the temperature is not much below 20 C. The movements in any case increase in energy as the blood is raised in temperature to 37 to 38 C [R. 210].*

General references to blood.—Many of these give numerous special references [B–S. 17; Heitz, 64; (Q. j. m. s. (1871) 361); Quain, II, 9, 23; R. 148, 178; St. 67; Str. 263; S 2. 7; Milne-Edwards, I, 49,83; Todd, I, 404. See also § 70 of these notes].

(§ 14) **Counting blood corpuscles.**—The average number in a cubic millimeter of healthy human blood is between five and six million red ones and from 40 to 140 thousand white ones. In the cold blooded vertebrates the red ones are less in number, and the white ones proportionately more numerous. For the methods of counting see [Str. 269; R. 203; S 2. 6; St. 86; Sat. 48].

(§ 15) **Protoplasmic movement in plants.**—In the cells of Chara, Vallisneria, Anacharis, etc., may be seen very clearly the movement of the protoplasm (*Cyclosis*). Take a leaf of Vallisneria, for example, shave off a thin slice, mount in water and observe with a 1–5 or higher objective. If the leaf to be used is kept in water at 25–30° C. for half an hour before making the preparation the movement will be more satisfactory (H. & M., 52).

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*A convenient warming stage may be prepared from a rectangular piece of sheet copper about the size of a slide. A perforation is made in the middle about the size of the opening in the stage of the microscope. To the rectangular piece is soldered a wire or narrow strip about 25 cm. long for a conductor. The warming stage is placed upon the microscope and the preparation upon it. The preparation is warmed by heating the conductor with an alcohol flame.

(§ 16) Infusoria and ciliated epithelial cells.—*Study and comparison of a ciliated unicellular organism, and a ciliated cell from a multicellular organism.*

Infusoria.—Infusoria are usually present in the aquarium containing amoebæ (§ 8). They are also abundant in water containing hay and standing in a warm place. To mount them, place on the slide some absorbent cotton, or preferably, some of the separated fibers of the absorbent paper used by dentists, and then press some of the wet hay or some of the vegetable matrial from the aquarium upon a cover-glass and mount upon the fibers placed on the slide; with blotting paper absorb part of the water around the edge of the cover. The fibers will confine the Infusoria so that they cannot get out of the field while one is observing them. Examine with 1–5 or higher objective [C. 497; H. & M. 89].

(§ 17) Ciliated epithelial cells.—To obtain these, open the mouth of a frog and scrape the roof with a scalpel blade. Mount the gelatinous substance so obtained on a slide in a drop of normal fluid, or in the absence of normal fluid, in a drop of spittle. Put a piece of hair in the preparation and cover. Press the cover-glass down quite firmly. If the preparation is to be studied for several hours, as in a class demonstration, seal it as directed for blood (§ 11). Examine with 1–5 or higher objective. If no cells are isolated, press directly down on the cover-glass to spread out the mass of cells. Make out the form of the cells, their ciliated ends (free ends), and the character of the ciliary movement. Compare with the movement of the cilia on the infusorian [Q. j. m. s. (1871) 308); Todd, I, 606, 628; Quain, II, 48; R. 240; F 1. 262; S 2, 24; B 1. 193].*

(§ 18) Anaesthetizing infusoria and ciliated cells.—Prepare a slide for this by cementing to it, with sealing wax, a glass tube drawn out somewhat at one end. The contracted end should be near the middle of the slide. Make a moist chamber with a ring of putty, in which is no turpentine, about the size of a cover-glass, and put it on the slide so that it shall enclose the free end of the glass tube. Make a furrow in the ring on one side, and place in it a drop of normal fluid or water. Place some Infusoria on a cover-glass and put the cover on the ring and press it down moderately. Place the preparation under the microscope and see that the Infusoria are active, then connect the tube opening into the moist chamber, by means of rubber tubing, with a wash-bottle. The tubes of the wash-bottle should both be short, and the one into which one blows should be connected with a rubber tube 40–50 cm. long so that one may blow and continue looking into the microscope. Put into the wash-bottle a little water and then two or

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*Ciliated cells may be obtained from the trachea of a snake, lizard or turtle, or from the gill of a clam or oyster, etc.

three cubic centimeters of ether or chloroform. Find an active infusorian that cannot get out of the field and then blow into the wash-bottle. The vapor of the anaesthetic will pass into the moist chamber, and will anaesthetize the infusorian. After the movement ceases remove the wash-bottle and blow air into the moist chamber. After a few minutes the movements will recommence.

Ciliated epithelium.—Scrape a frog's mouth as before (§ 17), and put the scrapings on a cover-glass with spittle or normal fluid, and put the cover-glass on the moist chamber as for the Infusoria. Get in view some active cilia and then blow into the moist chamber the chloroform or ether vapor as before. The cilia will gradually cease moving; remove the wash-bottle and blow air into the moist chamber. The cilia will resume their movement. The experiment may be repeated several times. *

CIRCULATION OF THE BLOOD.

(§ 19) **Circulation in the gills of Necturus.**—Select, if possible, a small animal with long gill filaments. Inject into the back three or four drops of *curara* solution (§ 76). Replace the animal in the water and in an hour or two it will be motionless. †

Place the Necturus dorsicumbent on the well moistened circulation board and cover it with a wet cloth.‡ Spread some of the gill filaments out on the glass cover of the perforated cork, using a well wetted quill duster. Place one end of the circulation board on the stage so that the gill filaments will be in the field. Use a diaphragm with a

*If the vapor is too concentrated, the movement ceases almost instantly. If it is continued too long, the movement will not recommence [S 2. 27; B–S. 35; Str. 5].

†A curarized Necturus should be kept in running water, in order that the respiration may not be interfered with. After a day or two the effect of the curara disappears. As curara paralyzes the motor nerves but is not an anaesthetic, no dissection should be performed without either destroying the brain (§ 9) or rendering insensible by an anaesthetic.

‡An excellent circulation board for Necturus and frog may be prepared by boring a hole about 2 cm. in diameter in a pine board 8x30 cm. and 15 mm. thick. The hole should be about 5 cm. from one end and near one side. A perforated cork or hollow cylinder of wood should be fitted to this hole. Over the top of the perforated cork should be placed a very thick cover-glass or a piece of thin glass slide, and sealed with sealing-wax; finally the whole board should be covered with woolen cloth or cotton flannel. The perforated cork should be capable of being moved so that it will stand a centimeter above the surface of the board if desired.

large aperature, and light with plentiful transmitted light. Employ a 3–4 objective and examine some isolated gill filaments. Demonstrate:

(1) Two large vessels, one on each side of the filament, making a loop over the free end.

(2) The blood flows in opposite directions in the two large vessels.

(3) The current alternately increases and diminishes. This indicates the heart beats.

(4) Besides the loop the large vessels make near the end of the filament, there is a capillary network connecting the two in all parts of the filament.

(5) Blood corpuscles are in all the vessels and are carried along by the current.

Put a cover-glass over some of the filaments and use a water immersion objective (1–10 or 1–15) if possible, and demonstrate:

(1) The capillaries are well defined channels.

(2) The flow in them is slower than in the larger vessels.

(3) The blood corpuscles are of two kinds: (a) Large oval colored ones which are very numerous. (b) Colorless or white corpuscles which are circular in outline or which are very large and appear in general form like the colored ones (§ 9).

(4) The corpuscles are elastic and flexible. This may be seen at some sharp angle where the corpuscles are often bent at right angles; they always resume their shape when free in the current. The nucleus is very apparent in most of them.

(5) Amoeboid movement in the white corpuscles may often be seen when the current in a capillary ceases for some time. Under favorable conditions, the amoeboid movement of the nucleus may also be seen in the white corpuscles so stranded (S. VII, 35).

(§ 20) **Circulation in the frog's foot** (interdactylar membrane).—Select a small frog if possible. The common spotted frog (Rana halecina) is a good subject. Curarize by making a small incision through the skin in the dorsal region with scissors, and introducing two or three drops of curara solution (§ 76) with a dropping tube. Put the frog in a moist, cool place, but not into water. In half an hour or more the frog will be motionless.*

Put the frog into a woolen sac well saturated with water. The sac should be only a little longer and wider than the frog's body. Leave one leg projecting. Pin the mouth of the sac, but do not pin tightly

* If the frog cannot be curarized it should be pithed, as described above (§ 12).

enough to compress the projecting leg. Place on the circulation board, being sure that the cloth covering the board is well filled with water.

Spread out the web by tying a thread around the longest dactyl, 4th, and one around the 3d or 5th and pinning the strings in such a position that the web shall be over the glass in the top of the cork. Do not stretch the web too tightly for it would be torn or the circulation impeded. Cover the exposed leg with a wet cloth or a piece of blotting paper. *Keep the web and the animal moist* (§ 19).

Put the circulation board on the stage so that the web shall be in the field. Employ the 3-4 objective and A ocular. Light as above (§ 19). Demonstrate the following points:

(1) The arteries give off branches and hence become smaller. The current flows from the larger part of the vessel toward the smaller part, and into the branches.

(2) The veins receive branches and hence become larger. The blood in them flows from the smaller part of the vessel toward the larger part, and from the branches into the large vessel.

(3) The branches given off by the arteries finally break up into an anastomosing net-work of capillaries. These capillaries unite and the vessels so formed are veins. This may be demonstrated, by moving the circulation board so that a branch may be followed.

(4) The stream is most rapid in the larger vessels; and sometimes pulsations corresponding to the heart beats may be seen in the arteries. Compare § 19 (3).

Remove the 3-4 objective and put in its place 1-5 or a higher one, a water immersion if possible (§ 19).

Cover the web with a triangular piece of cover-glass. Demonstrate the following points in addition to those already made out:

(1) Bring a vessel into the field that appears about 2 mm. in diameter. The central part of the stream is rapid, while that along the edges is slower. This part is sometimes called the inert layer.

(2) Focus sharply on the edge of the stream. In it will be seen many white corpuscles. These are mostly smaller than the red ones, and they roll along the walls of the vessel as if they were sticky.

(3) The central part of the stream is mostly composed of red corpuscles.

(4) Bring some capillaries into the field. The white and red corpuscles are mingled and go along in single or double file. The red

ones bend or elongate as conditions may require, showing that they are elastic and flexible, like those of Necturus (§ 20, 41).* † ‡

References [B 1, 191 ; B-S, 121 ; R, 598 ; St 158 F 1, 246 ; S 1, 154 (Ar. m. a., XIII, 483)].

PERMANENT PREPARATIONS OF BLOOD.

(§ 21) **Amphibian blood.**—A permanent preparation of amphibian blood, especially for the red corpuscles, may be prepared by spreading some fresh blood on a cover-glass upon which has been previously spread some normal fluid. Hold the cover-glass over the open mouth or in the neck of a bottle containing osmic acid (§ 77) for about one minute. The fumes will fix the corpuscles. After the corpuscles are fixed, place on the cover a drop of 75 per cent. glycerin containing alum carmine and fluorescine (§ 81) ; and then place the cover on the center of a clean slide, and allow it to settle down upon the slide by its own weight,—do not press it down.§

(§ 22) **Sealing the preparation.**—Place four drops of shellac cement (§ 84) at equidistant points around the cover-glass so that half the cement shall be on the slide and half on the cover. Allow the slide to lie flat for a few hours so that the cement may dry and fix the cover. Finally wipe away the glycerin around the edge of the cover-glass with a moist cloth, then place the slide on the turntable and make a ring around the entire cover-glass, putting half the ring on the slide and half on the cover-glass. Several thicknesses of the cement should be put on, but each one should be allowed to dry before adding another [*Seiler, 92*]. Any preparation mounted in a medium miscible with water may be sealed or cemented as just described.

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* It sometimes happens that the direction of the current is reversed. This is due to some obstruction, or to the contraction of some vessels and the expansion of others [R. 606].

† The dark irregular bodies seen in the web are pigment cells.

‡ If the current is too rapid to admit of the satisfactory demonstration of points (1 to 3) when the high power is used, control it by pressing the legs at the knee. This method, so far as is known to the writer, was devised by a laboratory student, C. E. Atwood, Cornell, '80.

§ Preparations may be placed in the center of the cover-glass very easily by preparing a card with circles upon it the sizes of ordinary cover-glasses, and cementing three slides or strips of bristol-board so that when the slide on which the preparation is to be made, is placed on the card its center will be over the center of the concentric circles made on the card.

(§ 23) Permanent preparation of extended white blood-corpuscles.—In the preparation described above (§ 21), the white corpuscles are mostly in an unextended condition. In order to moke preparations in which the corpuscles are fixed in the various phases of amoeboid movement, some of the pericardial or abdominal liquid should be obtained and placed on a cover-glass as directed in (§ 9–10). It should then be placed over a moist chamber made of a ring of putty on a slide and containing a drop of normal fluid. This preparation should be examined occasionally and when the white corpuscles are satisfactorily extended (after half an hour or more), the cover is grasped by the fine forceps and held in or over the neck of a bottle containing osmic acid solution (§ 77) for about a minute. Then a drop of alum carmine is put upon it and allowed to remain for a minute or two. Finally, after removing part of the alum carmine, with filter paper, a drop of the colored glycerin is added as above (§ 21), and the cover is put on a slide, sealed, etc., as described in (§ 22). In successful preparations, both red and white corpuscles are present; and the white ones show various amoeboid phases both of cell-body and nucleus [(Q. j. m. s. (1871) 371), (S. VII, 35)].

(§ 24) Permanent preparations of mamalian blood.—(1) *Moist preparations* may be made as described in (§ 21). The white corpuscles are usually circular and their nuclei are in many cases stained by the alum carmine. To render more certain the staining of the nuclei of the white ones, a drop of pure alum carmine may be used immediately after the treatment with osmic acid as described in (§ 23).

(2) *Dry preparations.*—The blood to be mounted should be as fresh as possible; and if it is from an animal it should be free from foreign material. Human blood may be obtained as directed above (§ 13). The blood should be spread on the cover-glass in a layer or film so thin that the corpuscles are not in contact. To do this, select a slide to spread the blood whose edge is straight and rounded like the base of the letter U. Touch the edge of the spreading slide to the blood, press it gently against the cover-glass and draw it quickly across the cover. If the room is warm and dry the film will dry very quickly; if not, warm the cover over an alcohol lamp. Examine under the microscope, and if the preparation is satisfactory, mount as follows:*

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*It is nearly impossible to make a preparation which shall be equally good in all parts, hence if some parts of the preparation are satisfactory it should be retained.

† Preparations of spermatozoids, and many other bodies free in a liquid may be mounted dry as described for blood. By heating to 50–60 C. so that the film will not wash off, various stains may be used. After the preparation is stained it may be mounted dry as for blood, or in balsam, glycerin, etc.

Make a shallow cell of shellac or other cement of about the size of the cover-glass, in the center of a slide. Let this dry spontaneously, or if in a hurry, use heat. When the cement is dry, warm the slide and the cover-glass on which is spread the film of blood, and put the cover on the cell, film side down. Press the cover-glass against the cell with the convexity of the tracer or fine forceps, until there appears a shining or polished appearance all around the cell. This indicates that the cover adheres to it. Seal the cover-glass as directed above (§ 22).

Do not breathe on the slide or cover-glass in any of the operations; for even a slight moisture will ruin the preparation in a short time. [C. 713; R. 195; F 1, 218, 240; (Ar. m. a. I, 340) VIII 476 and XIII, 753) *Wormley* 740; *Woodward*, The application of photography to micrometry, p. 4].

§ 25) Storing, labeling and cataloguing microscopical preparations.—(1) *Storing*.—Microscopical specimens should lie flat and be protected from dust and light. (2) *Labeling*.—Each specimen should be labeled, and the label should give, (a) the number, (b) the date, (c) the general name, and (d) the source of the preparation; and (e), if possible, the thickness of the cover-glass.*

(3) A catalogue of each preparation should be made, and should contain a summary of the history of the specimen, including the methods of preparation. It is only by the possession of such knowledge that the comparative excellence of methods can be determined, and the defective ones discarded or improved.†

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* It is desirable to know the exact thickness of each cover-glass used for two reasons: (1) One need then never through ignorance use so thick a cover that high objectives cannot be employed in studying the preparation; (2) The perfect adjustment for the cover-glass in adjustable objectives is greatly facilitated by knowing the exact thickness of the cover-glass in each case. This is especially true of objectives like those of Zeiss, in which the adjusting collar of the objective is marked with numbers corresponding to cover-glasses of various thickness [A. m. s. (1884), 202].

† (1) The catalogues are made on thick slips, postal card size, and arranged alphabetically in a box as is commonly done in a library slip-catalogue. (2) The labels are prepared from linen paper of moderate thickness. They are gummed with liquid gelatine (§ 86) and attached simply by wetting them as in using postage stamps. (3) The ink employed is Higgins' *water proof India ink* diluted with an equal quantity of soft water to which has been added a few drops of ammonia.

Formula for cataloguing Microscopical Preparations :—

1. General name, and source.
2. Number and date of the preparation, and the name of the preparator.
3. Special name of the preparation ; the common and scientific name of the object from which it is derived.
4. Special object of the preparation.

5. Chemical treatment, — the method of hardening, dissociating, etc.
6. Mechanical treatment,—imbedded, sectioned, dissected with needles, etc.
7. Staining agent and the time required for staining.
8. Clearing agent, the mounting medium and the cement used for sealing.
9. Objectives to use in studying the preparation ; thickness of the cover-glass.
10. Remarks, including references to good figures and descriptions.

A Catalogue Card Written According to the Formula :—

1. Myelinic nerve-fibres. Cat.
2. No. 31 (Dr'r. II), March 21, 1880 ; S. H. G.. preparator.

3. Isolated, myelinic nerve-fibres from the sciatic of the cat (*Felis domestica*).

4. This preparation shows well the axis cylinder and the nodes of Ranvier.
5. Dissociated 24 hours in 35 per cent. alcohol.

6. Dissected on the slide with needles.

7. Stained over night (12 hours) in picrocarmine.
8. Cleared with turpentine and carbolic acid ; mounted in chloroform balsam ; sealed with shellac.
9. Use three-fourths and higher objectives. Cover .15 mm.

10. See for figures and descriptions (Quain's Anatomy, Vol. II., p. 141, and Ranvier, Traité d'Histologie, p. 723).

Formula for Labeling Microscopical Preparations :—

Number and date of the preparation (No. 2 of catalogue).
General name (No. 1 of catalogue).
Name of the object from which the preparation is derived.
Thickness of the cover-glass.

A Label :—

No. 96 ; March 12, 1880.

Myelinic nerve-fibers.

Cat.

Cover .15 mm.

[(A. s. m. (1883) 169), (J, r. m. s. (1883) 924). (Z. w. m. (1884) 280.) Beh. 245.]

ISOLATION AND PRESERVATION OF THE STRUCTURAL ELEMENTS.

(§ 26) **Ciliated epithelial cells.**—Soak the trachea of a cat or other animal, or the oesophagus or roof of the mouth of a frog in 35 per cent. alcohol from one to three days, preferably but one day. Transfer to water, and scrape the mucous surface with a scalpel or needle.

Place the substance so obtained on the center of a slide and spread it somewhat with needles. Add a drop of fluorescine. After a minute remove the fluorescine, but do not wash with water. Finally smear a cover-glass with alum carmine glycerin and cover. Press the cover-glass directly down quite firmly. Isolated, ciliated cells should be found in abundance. Seal the preparation as for blood (§ 22) [(Ar. m. a. XI, 354) and as in § 17].

(§ 27) **Striated muscular fibers.**—Select a muscle with parallel fibers,—a ribbon shaped muscle like the *occipito-scapularis* [W. & G. 217] answers very well. In obtaining the muscle, wet the hair thoroughly before cutting the skin, to avoid hair on the tissue. After the muscle is exposed cut one end and grasp it with forceps. Hold the muscle tense, isolate and remove it either with scissors or a sharp scalpel. Lay the muscle on a strip of cork and put a ribbon pin through each end and into the cork. The muscle, and also other tissues, should be about as tense on the cork as in the body, that is they should be in a state of *physiological extension* [R. 729]. After the muscle is pinned to the cork be sure that it does not touch the cork, then invert it in 35 per cent. alcohol. In from one to three days transfer to water, and cut out a piece about 5 to 10 mm long and 2 mm in diameter, and transfer it to the center of a slide. Place over it a tripod magnifier and with a clean, bright needle and the fine forceps or two needles, separate the fibers at one end so that the piece of tissue shall appear fan-shaped. To do this, grasp the tissue at one end with the forceps or hold it down with a needle. With a needle remove all the connective tissue as far as possible. The final success of preparations of isolated tissues depends largely on the thorougness with which this is done. After the connective tissue is removed, fray out the fibers at one end by drawing the needle lengthwise something as if combing. It is often necessary to use both needles on a fine bundle and draw the fibers apart. This is usually best done after a pretty thorough longitudinal combing. It is usually not best to try to isolate the fibers the entire length of the piece; for the final result is usually more satisfactory if the fibers are more or less combined at one end. After the isolating is judged to be sufficient, a drop of alum carmine or haematoxylin (§ 87, 88) should be put upon the tissue and allowed to remain from one to five minutes. This should then be washed off with water, and a drop of fluoriscine added. After a minute or two the fluorescine is washed off and then the preparation may be mounted in glycerin as for the ciliated cells (§ 26) or preferably Canada balsam (§ 93) may be used (§ 27a) [Quain II, 118; R. 468; Heitz. 265; (A. s. m. (1882) 131); (Q. j. m. s. (1885) 371); Leydig 2. 124].

(§ 27a) **Mounting in Canada balsam.**—(1) The water must be removed without allowing the object to become desiccated; this is usually accomplished with 95 per cent. or stronger alcohol. (2) The alcohol is replaced by the clearing agent—carbolic acid and turpen-

tine, clove oil, etc. (§ 94), which must be miscible with the balsam. (3) The clearing agent is replaced by balsam.

To mount the muscle in balsam, put upon it several drops of 95 per cent. alcohol with a dropping tube. After a minute allow the alcohol to drain away, and then add a drop of the clearing mixture (§ 94). While this is acting, arrange the specimen and separate the fibers still more if possible, then remove the clearing agent from around the tissue, and place upon it a cover-glass previously spread with balsam. Air bubbles will soon disappear. Balsam preparations need not necessarily be sealed. If it is desired to seal them, wait till the balsam has hardened around the edge of the cover, then put on two or three rings of shellac as in (§ 22) [Fl. 210; R. 138].

(§ 28). **Muscular-fiber Cells.**—*Plain, unstriated or smooth muscle. Contractile fiber-cells.* To obtain these remove a small piece of the muscular wall of the stomach or intestine of any animal—or take the muscular wall of the urocyst or any other organ where muscular fiber-cells are known to exist, and place it in 15 to 20 cc. of 20 per cent. nitric acid (§ 78) for three days. Then pour off the acid and soak an hour or more in water, or 35 per cent. alcohol. [Reichert's method, Kl. 89]. Place the tissue in a homœpathic vial about half filled with water and shake the bottle. The cells will separate. Allow the bottle to stand half an hour for the cells to settle, then remove most of the liquid, and add 5 to 10 cc. of alum carmine. Allow the stainer to act an hour or more, then remove most of it, fill the bottle with water and as soon as the cells have settled, remove most of the water and fill the bottle about half full with fluorescine glycerin (§ 82). Mix the cells with the glycerin by rolling the bottle. Shaking would fill the glycerin with air bubbles. To mount a preparation, place a drop of the mixture on the center of a slide and seal as for blood (§ 22). Muscular fiber-cells may be isolated by using needles after dissociating a day or two in 35 per cent. alcohol, but it is difficult to find unbroken fibers. The cells are quite well stained in picrocarmine or haematoxylin.* [Fl. 318; Q. 530; Ru. 90; Sl. 112: Kl. I, 89; Ar. a. e. (1849), 180).

(§ 29). **Cardiac muscular fibers.**†—Cut from the surface of a ventricle a thin section in order to remove the proper serous covering of

* Picrocarmine usually stains muscular fiber-cells well after they have been isolated in nitric acid. Haematoxylin should not be used as it is not a permanent stain for objects containing an acid. It answers well, however, for the cells isolated by means of alcohol.

† The capillaries and small vessels of a cat's heart are usually full of blood. The corpuscles are rather confusing in the final preparation, hence the blood should be washed out. To do this ligate the subclavian and brachiocephalic arteries [W. & G. 350], and inject salt solution into the aorta toward the heart (§ 74). The semilunar valves will close and the liquid will enter the cardiac arteries. When the ventricles look pale the injection may cease.

the heart. Make a second section, partly tearing it with the fingers, in order to get the fibers in their length. Place part of the sections so obtained in 20 per cent. nitric acid over night, and part of them in 35 per cent. alcohol. The branching net work of fibers may be most easily demonstrated by using nitric acid, but the nuclei are difficult to stain after the acid treatment. They are easily demonstrated in the sections dissociated in alcohol, however.

After about 12 hours remove the sections from nitric acid, and soak them in a plentiful supply of water for an hour or more; a day or more in water, or preferably in 35 per cent. alcohol is advantageous. When ready to make the preparation, place a small piece on a slide and carefully and thoroughly separate the fibers with needles. Use the tripod magnifier. The fibers can hardly be too thoroughly isolated. They cannot be combed out longitudinally as in striated muscle, but have to be pulled apart laterally or transversely using two needles. After the tissue is well teased or dissected, add a drop of alum carmine, and allow it to remain 5 minutes or longer, then wash it away carefully and add fluorescine. Allow the fluorescine to remain 5 minutes or more, and then wash it away. Finally, dehydrate by adding alcohol and mount in balsam, as described (§ 27a). The sections dissociated in alcohol should be teased with needles, stained and mounted as just described. Quain, II., 135; Str. 179; R. 539; *Gibbs.* 74; *Prudden*, 101; Ki. II. 281. (Ar. m. a. XXIII, 500.)]

(§ 30) **Myelinic nerve-fibers.**—(*White, medullated or dark-bordered nerve-fibers*).—These may be obtained from any of the nerves of the limbs. The sciatic nerve is probably the best to use. The nerve should be carefully removed from the body and pinned on a strip of cork and placed in 35 per cent. alcohol as described for striated muscle (§ 27). After remaining in the alcohol from one to three days it should be treated exactly as for striated muscle. Special pains is necessary in removing the connective tissue, and as the fibers are smaller than those of muscle, the teasing must be carried further. When the isolation is deemed sufficient, a drop of alum carmine is added and allowed to remain from three to five minutes. This is then washed away and a drop of fluorescine allowed to remain half a minute or a minute. The tissue is then washed with water and mounted in glycerin or preferably in balsam as directed in (§ 27a) [S. 710; *Schwann* 166; Sc. 290; Sat. 180; Str. 120; Quain, II, 138].

(§ 31) **Amyelinic nerve-fibers.**—(*Non-medullated, gelatinous or gray nerve-fibers, fibers of Remak*). In demonstrating amyelinic nerve-fibers it is best to select a nerve in which are also present myelinic fibers in considerable abundance. A splanchnic nerve [W. & G. 395] is perhaps best. A piece of the sympathic [W. & G. 395] or the ulnar or musculo-cutaneous nerve [W. & G. 386, 384] answers very well,

also. The nerve should be dissociated in 35 per cent. alcohol, as described in (§ 27, and 30). The amyelinic nerve-fibers are, in the cat, usually smaller than the myelinic, and the teasing must be very thorough. The use of two needles for drawing the fibers apart after combing or fraying the fibers longitudinally is recommended (see also § 29). The staining and mounting should be as described in (§ 27, 27a and 30).* [R. 746; Quain, II. 145; Sc. 290; F. & L. 72; Str. 121; Klein, 111; *Schwann*, 169, 170].

(§ 32) **Areolar tissue.**—This is most satisfactorily prepared by forming an artificial œdema (Ranvier's method, R. 329): Place a cat dorsicumbent, and fasten the legs laterad with cords. Thoroughly moisten the hair along the cephalic (inner) aspect of the leg, and after parting the hair, make an incision through the skin parallel with the femur, extending the incision slightly beyond the knee. Tear the skin from the muscles for a short distance, and there will appear a delicate fibrous substance holding the skin to the muscles. This is the areolar or loose connective tissue, composed in part of white fibrous and in part of elastic or yellow fibrous tissue and a variable number of connective-tissue corpuscles. With a syringe or a pressure bottle, (§ 37) inject into the tissue picro-carmine or alum carmine. In doing this, insert a small, sharp pointed connula for some distance into the tissue and continue the injection until an œdema or swelling of considerable size is formed. After 15 or 20 minutes remove the rather thick layer on the surface of the œdema with scissors, and then cut as thin a piece from the underlying tissue as possible. Scissors should be used which are sharp. This small mass will appear almost like jelly. Transfer it to a watch-glass of distilled water. Place on the center of a cover-glass a drop of fluorescine glycerin (§ 82), and put the cover where it may be easily grasped by the fine forceps. Transfer the tissue to the center of a slide, and with blotting or filter paper absorb the liquid around it. spread the tissue as thinly as possible upon the slide. Do this under a tripod magnifier. Use a pair of bright dissecting needles and grasp opposite edges of the tissue and draw them as far apart as possible without tearing. If there is not too much liquid on the slide the edges will cling to the slide as they are drawn out and thus enable one to spread the tissue very thinly. One should work rapidly, and if the tissue is in danger of drying it

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*A very striking and instructive preparation may be made by placing the nerve for 12 to 24 hours in a one-twentieth per cent. osmic acid solution. The osmic acid blackens the myeline of the myelinic fibers. It is also a dissociating fluid, so that the nerves may be easily isolated with needles after its action. If the preparation is teased and treated exactly as above, the myelinic fibers will appear black or dark gray, the axis cylinder being more or less reddened, while the amyelinic fibers will be red.

should be breathed upon. As soon as the tissue is properly extended, the cover-glass should be applied. The preparation should be sealed as described in (§ 22). In preparing areolar tissue it is very desirable to use a kitten or other young animal, for the white fibrous tissue is neither in such large bundles nor so greatly in preponderance over the elastic tissue. The connective-tissue corpuscles are also more numerous. If the edge of a fat tract is included in the œdema, one is very likely to obtain, in addition to the connective tissue proper, a network of small blood-vessels and capillaries with fat cells in the meshes. The fat cells are often in various stages of development—the so-called signet-ring stage being the most common [*Schwann, 140*]. Excellent preparations of areolar tissue may also be made by injecting serum (§ 73), salt solution (§ 74), or even water into the tissue instead of the coloring matter as described above. Pieces of the right size are cut out with scissors, and stained 10 to 30 minutes in alum carmine, then for five minutes or more in fluorescine, and mounted in alum carmine glycerin as above. [R. 329; S1, 72; Quain II, 61; F1, 274; (J. r. m. s. (1879) 355); (Ar. m. a. XI, 176; XII, 391; (Ar. a. e. (1879) 401); A. s. m. (1882) 109)].

(§ 33) **Endothelium.**—(*The epithelium of serous membranes*). To demonstrate endothelium, kill a Necturus by pithing, or by putting it in chloroform or alcohol in a small amount of water. Open the body cavity by a longitudinal incision 1 cm to the left or right of the ventrimeson. A transparent ligament will be seen extending from the ventrimeson to the liver. Remove a piece of this with scissors and place it in a watch-glass of water. Force a stream of water upon it from a wash-bottle to wash away any lymph or lymph-corpuscles from the surface. Remove the water, and spread out the membrane on the bottom of the watch-glass. Pour upon it a one-half per cent. solution of silver nitrate, and allow it to remain a minute or two. Then pour off the silver and wash well with water. Finally place the watch-glass containing the membrane in a plentiful supply of water in a strong light. As soon as the membrane has turned brown, pour off the water, spread out the membrane and examine. If the cell outlines are indicated by dark lines, the staining is sufficient; if the outlines are not distinct continue the exposure till they are. It should be remembered that there is a layer of cells on both surfaces of the membrane. When the silver staining is sufficient, transfer a piece of membrane of the proper size to the center of a slide; cover it and put a spring compressor upon it to hold the cover in place; then put the preparation in a jar of 95 per cent. alcohol over night. The cover may then be removed and the membrane washed with water and stained with a drop of alum carmine or haematoxylin for a minute or two, then washed and stained a minute or more with a drop of fluorescine. After the fluor-

escine is washed away with water, the membrane is dehydrated by dropping on it alcohol with the dropping tube (§ 27a). It is then cleared and mounted in balsam as directed in (§ 27a). This method of mounting membranes in balsam was first suggested, so far as is known to the writer, by the late Dr. Woodward [L. (1872) 99, 163].

To avoid the wrinkling or folding that is almost sure to occur in the ordinary method of transferring the membrane to the slide, a dish of considerable size should be used, and the slide partly or wholly immersed so that the membrane can be floated upon it [(L. I, 158); (Z. w. m. I. 392); (A. m. m. j. VI, 235; VII, 13); (J. d. a. p. XII, 54, 588); (J. r. m. s. (1879), 357); R. 105, 246; Quain, II; K. 89; B-S-43; K. & S. 180].

(§ 34) **Cartilage.**—An excellent source for hyaline cartilage is an end of one of the long bones of Necturus. The larynx of the cat also furnishes good material. Sections may be made free-hand of the fresh material as follows: Remove most of the soft parts covering the cartilage, wrap it in newspaper or other strong paper, and grasp it by the thumb and index of the left hand so that it projects slightly above the nail of the thumb and index. Wet the cartilage well with salt solution (§ 74) and wet the razor or section knife with the same. Grasp the razor firmly, rest the blade on the nail of the thumb or index; move the razor forward across the tissue with a drawing cut, making each section with a single sweep of the knife. Make several sections, and transfer them to a dish of picric acid solution (§ 83). They should be left in this half a day or more. They may then be washed with water and mounted in glycerin or glycerin jelly (§ 80). If it is desired, the sections may be stained with alum carmine after soaking in water till the picric acid is removed. Stained sections may be mounted in balsam, but glycerin or glycerin jelly is preferable. As it is sometimes impossible or undesirable to make sections of fresh cartilage, the cartilage in small pieces may be kept in the picric acid for a few days and then in 75 to 80 per cent. alcohol until one is ready to make the sections. In that case the sections are transferred to water or 35 per cent. alcohol as they are cut, and the razor and tissue are wet with the 35 per cent. alcohol, with salt solution or with water. The sections must not be allowed to dry in any case. It is difficult for the inexperienced to make free-hand sections of sufficient thinness and evenness, hence the cartilage may be cut in the microtome as follows: Fill the well of the microtome nearly full of melted imbedding mass (§ 103); dry one end of the cartilage with filter paper, and insert it into the melted mass so that the other end of the cartilage is about on a level with the top of the microtome. To prevent drying, place a small mass of absorbent cotton, wet with salt solution, over the exposed end of the cartilage. Cool the imbedding mass as

soon as possible by the use of snow, ice or cold water. Wet the razor with salt solution and make the sections as rapidly as possible, using a drawing cut as in free-hand sections. (See also § 40; 58).* [R. 274; Quain, II, 77; Heitz. 198; Sat. 82; Str. 83; K. 45; *Leydig* 2, 73].

HARDENING TISSUES.

(§ 35) **Hardening agents.**—A great many agents are used for hardening and preserving tissues for histological purposes. Those most used are alcohol, chromic acid and various salts of chromium, as dichromate of potassium and ammonium. Picric acid is also often employed. None of them are equally good for all tissues, and in most cases two or more of them are employed on each tissue. A perfect hardening and preserving agent for histological purposes would leave the structural elements with exactly the appearance they possess during life, color perhaps excepted.

Fifty to seventy-five (50–75) times as much liquid should be used as tissue, and if the tissue is of a massive character, like liver, only two to three cubic centimeters should be taken. **Label everything.** *Put the tissues into the hardening agent as soon as possible after the death of the animal.*

(§ 36) **Hardening in picric acid solution.**—(§ 97).—Nerve (12), striated muscle (14), trachea (16), stomach of Necturus (18), ileum (19), and ovary may be hardened in picric acid solution as follows: Fasten a piece of sciatic nerve and of the occipito-scapularis muscle, obtained as directed in (§ 27, 30), to a strip of cork and in physiological extension by wooden pegs—pins would be corroded by the picric acid. Invert the tissue in a vessel of the solution. Suspend the trachea, stomach, ileum and ovary in the solution by a string tied to some of the adherent connective tissue. In from one to three (1 to 3) days pour off the picric acid solution and add 95 per cent. alcohol. This should be changed once or twice a day until the alcohol is but slightly stained with the picric acid.

(§ 37). **Hardening in Müller's fluid** (§ 96).—Myel, artery, submaxillary gland, liver and human skin may be hardened in Müller's fluid as follows: The artery should be pinned out on cork as described in

* So far as I know this method of cutting cartilage in a microtome, without complete imbedding (§ 39) was first suggested by B. L. Oviatt, Cornell, '87. The cells of cartilage are very liable to shrink and appear quite unlike those of fresh cartilage if the ordinary methods of preservation are employed. The picric acid solution has been found very satisfactory for all kinds of cartilage.

(§ 27) and inverted in the liquid. Myel, submaxillary gland, liver and human skin may be laid on a bed of absorbent cotton and the liquid poured upon them. This is practically suspending them in the liquid. The Müller's fluid should be changed at least twice, viz.: on the 2d and 4th days after the tissues were put into it. After the tissues have remained in the fluid for 12 to 14 days, pour it off, wash the cotton thoroughly or use new cotton, and place the tissues in 50 per cent. alcohol for one or two days, then into 75 per cent. for the same time, and finally into 95 per cent. alcohol till ready to make sections [F. & L. 245; S1 and S2.; Ru.; F1.; B-s.; Stowell; R.; Heitz.; Sat.; Lee; W.; Fol.; Car.; Str.; Tr.; Beh.; (J. r. m. s.); (Z. w. m.)]. *

IMBEDDING AND SECTION CUTTING.

(§ 38) **Infiltrating with paraffin.**—At the present day most objects to be imbedded in paraffin are previously infiltrated or thoroughly permeated with it so that all the cavities of the tissues are finally filled with paraffin. To do this: (A) All the water must be removed from the tissue by means of 95 per cent. or stronger alcohol. (B) The alcohol is replaced by chloroform by placing the tissue in a wide mouth bottle with 10 to 15 times as much chloroform as tissue. After 12 to 24 hours the tissue is usually thoroughly permeated with chloroform. A longer stay in the chloroform does no harm. (C) The tissue is placed in a mixture of paraffin 4 parts chloroform 1 part, which is kept just melted, the temperature never being allowed to exceed 55 or 60 C. The tissue should remain in this from 12 to 24 hours and then (D) It is placed in pure paraffin (§ 103) kept just melted, and allowed to remain from 12 to 24 hours.

(§ 39) **Imbedding.**—After a tissue is infiltrated it should be imbedded in pure paraffin as follows: A small paper box, made of a piece of strong paper 4x7 cm, is nearly filled with melted imbedding mass, and the tissue is placed in it and quickly arranged, so that the object will be cut at the proper angle by cutting at right angles to the length of the box. As soon as a film is formed by the cooling of the imbed-

* An excellent method of hardening tissues is to inject the hardening agent into the arteries, going to the tissue or into the ducts of glands. This may be done for all tissues and for all hardening agents by boring two holes in the top of a preserving jar, and making a kind of wash-bottle of it. Pressure may be obtained by using a constant pressure apparatus, or an atomizer bulb. The connection with the vessel or duct may be readily made by a rubber tube and glass cannula. This form of injecting apparatus is especially adapted for corrosive liquids like picric, and osmic acid, and silver nitrate, etc.

ding mass the box may be placed in cold water or in any cold place so that the paraffin will cool quickly and avoid air bubbles. It is well to indicate on the box the name of the tissue and the end where the cutting should commence. The tissue so imbedded and labeled may be put away until one is ready to make sections of it.

(§ 40) **Sectioning.**—When one is ready to make sections of a tissue it is put into the holder of a microtome. If the microtome is the well form, the screw is lowered until the imbedded tissue, from which the paper box has been removed, will reach the top of the microtome. The end of the imbedding mass is cut away until the tissue is visible, then it is placed in the well of the microtome and melted paraffin poured in to fill the well. The paraffin should be hot, 65 or 70 C, so that it will melt the surface of the imbedding mass containing the tissue and become one with it. After the paraffin is cool, trim it away from the tissue so that only a thin layer supports the tissue on every side. The appearance of the imbedded tissue when it is ready to be cut is something like a bluntly sharpened lead pencil, the sharpened end being square or rectangular in outline. In making the sections, if the edge of the knife is made parallel with one edge of the imbedding mass, consecutive sections will adhere to each other and form a kind of ribbon (see also § 60).*

If the imbedding mass is loose in the microtome, it may be made firm by crowding a thin wedge of wood between the wall of the microtome and the imbedding mass. This must not be allowed to project above the top of the microtome, however.

In making the sections, carry the knife directly across the tissue. In dry sectioning, as this is called, the more direct the cut the more successful the section ; and usually a very rapid movement gives the best results. *Perfect sections should consist of but a single layer of structural elements.* The sections may be preserved for an indefinite time without deterioration ; and they may be stained and mounted at any time (§41–46). For wet sectioning see (§ 34 and 58).

(§ 41). **Fastening the sections to the slide.**—In order that no part of a section shall get lost or disarranged during the staining and mounting processes, the sections should be fastened to the slide. (See also § 60). To do this a thin coating of collodion or celloidin (§ 101, 104) is put upon the center of the slide with a brush and allowed to dry. The sections are arranged on the slide and pressed down gently if they are not flat, and then several drops of ether and alcohol (§ 105) are put upon them with a dropping tube. The ether and alcohol soften the collodion, and the sections are cemented to the slide

* One may imbed directly in the well of the microtome and not use the paper box, but it is not so satisfactory.

by the collodion. After the ether and alcohol have evaporated (in 3 to 5 minutes), the slide on which the sections are fastened, is placed in a jar of xylol (§102) to dissolve out the paraffin. This will require about half an hour. The sections may remain in xylol for several days, perhaps indefinitely, without being injured. Before the sections are placed in xylol they may be exposed to the air without injury (§ 40), but after the paraffin is removed from them they would become desiccated if exposed to the air. * †

(§ 43). **Staining the sections.**—After the paraffin is removed from the sections by the xylol, the slide bearing them is placed for a minute or longer in 95 per cent. alcohol to wash away the xylol. The sections are then washed with water by a gentle stream from a wash-bottle. The stainer, if it acts within 1 to 20 minutes, is placed directly upon the sections and the slide laid flat. If a long time is required to complete the staining, place the slide in a jar containing the stainer. After the sections are stained sufficiently the superfluous dye is removed by a gentle stream of water.‡

(§ 44). **Dehydration.**—After the stainer is washed away, the sections are placed in a jar of 95 per cent. alcohol to remove the water. The sections may remain in the alcohol a considerable time without injury. They *should* remain in the jar five minutes or more, or the dehydration may be hastened by moving the slide or by pouring alcohol from a bottle over the sections.

(§ 45). **Clearing.**—The dehydrated sections are prepared for the final mounting in balsam, by putting upon them a few drops of clearing mixture (§ 94). The clearing is usually completed in two or three minutes or even less.

When sections are cleared they can hardly be seen if held over some dark object. If, on the other hand, they are held up between the eye and the light, they appear very translucent.

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* This method of fastening sections to the collodionized slide by using ether and alcohol, was first suggested in the laboratory, so far as I know, by H. E. Summers, Cornell '86. See also § 58 for fastening celloidin sections to the slide.

† The method originally suggested for fastening sections to the slide is as follows : Collodion (§ 101) is mixed with three to four times its volume of clove oil, and some of this mixture is brushed on the slide just before using it, or the collodion may be put upon the slide as described above (§ 41) and just before putting the sections on the collodionized surface it is lightly brushed with clove oil. In either case the slide, after the sections are upon it, is heated to 35—40 C. for a few minutes to drive off the clove oil. After the clove oil is expelled the sections are treated precisely as described in § 41. (See references to § 60).

‡ Although the sections may appear greasy when washed with water, the stainer will act if the paraffin was removed as described in (§ 41).

(§ 46). **Mounting the sections.**—As soon as the sections are cleared, the clearing agent shonld be removed with blotting or filter paper, then a cover-glass is spread with balsam (§ 93), and placed over the sections as in § 27a. For references see (§ 60).

ORDER OF PROCEDURE IN MAKING HISTOLOGICAL PREPARATIONS BY THE PARAFFIN METHOD.

1. Dehydrating the tissue with 95 per cent. alcohol (§ 38).

2. Soaking the tissue in chloroform (§ 38).

3. Infiltrating with paraffin and chloroform (§ 38).

4. Infiltrating with pure paraffin (§ 38).

5. Imbedding (§ 39).

6. Sectioning (§ 40).

7. Cementing the sections to the slide with collodion (§ 41).

8. Removing the paraffin from the sections with xylol (§ 42).

9. Removing the xylol from the sections with alcohol (§ 43).

10. Removing the alcohol from the sections with water (§ 43).

11. Staining the sections with a nuclear dye (§ 43).

12. Washing away the superfluous dye with water (§ 43).

13. Staining with a general dye (§ 43).

14. Washing away the superfluous dye with water (§ 43).

15. Dehydrating the section with alcohol (§ 44).

16. Clearing the sections with the clearing mixture (§ 45).

17. Mounting the sections in Canada balsam (§ 46).

18. Sealing the cover-glass(§ 27a).

19. Labeling the slide (§ 25).

20. Cataloguing the preparation (§ 25).

(§ 47) **Transection of a nerve.**—The sciatic is to be preferred. Arrange the nerve in the imbedding box and then in the microtome so that the sections shall be exact transections. Harden, etc., as in § 36, 38–42.*

Stain with carmine, or picro carmine, 15 to 20 minutes, or with alum carmine 5 minutes, fluoriscine 20 seconds. *References:* Same as in § 30, and R. 740, 756; Sc. 804; K. 103; Quain, II, 150; S2. 73].

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* Any organ or tissue may be cut across, or parallel with its own long axis without regard to the body axis. Sections at right angles to the long axis are called transverse sections, or more shortly *transections.* Sections parallel with the long axis are longitudinal sections, or more shortly *longisections.* See also § 60.

(§ 48) **Myel** (*Spinal cord*).—The cervical or lumbar enlargement is to be chosen. Imbed so that an exact transection can be made. If the nerve-roots and the ganglia are left on the myel, and held at right angles to it by the use of pins during the imbedding, the sections, when at the proper level, will include both nerve-roots and ganglia. For hardening, cutting, etc., see § 37–42.

Stain 15 to 20 minutes with carmine or picrocarmine, or 5 minutes with alum carmine and 15 to 30 secs. with fluorescine. *References:* Quain II, 269; S2 185; K. 127; Fl. 348; Sat. 298; F. & L. 212; Sc. 335; Str. 623; St. 242; W. 48, 59; Lee, 361].

(§ 49) **Transection of a striated muscle.**—The occipito-scapularis or other ribbon shaped muscle should be chosen. Arrange the muscle in the imbedding box and in the microtome so that exact transections can be made. For hardening, cutting, etc., see § 36, 38–42.

Stain 5 minutes with haematoxylin (§ 88), and 15 to 20 secs. with fluorescine. *References:* R. 496; K. 69; St. 136; Fl. 319; and as in § 27.]

(§ 50) **Transection of an artery.**—One of the arteries of the limbs,—femoral, brachial, etc., should be chosen for the muscular type of arteries. The aorta gives excellent preparations if the elastic type of arteries is desired. Arrange the artery for exact transactions. The hardening, sectioning, etc., are as in § 37–46.

Stain the sections with haematoxylin 3 to 5 minutes, or alum carmine may be used. *References:* R. 552; Str. 194; Sat. 151; Kl. II, 294; Quain II, 183; (J. d. a. p. (1865) 536)].

(§ 51) **Section of trachea.**—Longisections or transections may be made. If longisections are to be made, the trachea should be cut lengthwise and pinned out flat during the hardening and imbedding processes, otherwise the sections are liable to be oblique. Harden, imbed and section as directed in § 36, 38–46.

Stain 5 minutes with hæmatoxylin and then 15 to 30 seconds in fluorescine. [*References:* Kl. II, 165; Quain, II, 511; K. 217; Str. 435; *Todd*, V, 260; *Allen*, 689; Heitz. 709.]

(§ 52) **Section of submaxillary gland.**—Harden the submaxillary gland, as directed in § 37. Excellent preparations may also be made by hardening in picric acid and alcohol, or simply in 95 per cent alcohol. The hardening should commence as soon as possible after death. Imbed, section, etc., as in § 39 to 49.

Stain 3 to 5 minutes with haematoxylin, and then 15 to 30 seconds with fluorescine. Picrocarmine stains fairly well also. [*References:* Quain, II, 578; S2. 136; F. & L. 110; Str. 294. (Ar. m. a. XIII, 281, 359)].

(§ 53) **Section of stomach of Necturus.**—The most satisfactory preparations are obtained by injecting 95 per cent. alcohol, or picric acid and alcohol (§ 36) into the dorsal aorta before the removal of the stomach from the animal. The stomach should be cut lengthwise and placed immediately in 40 to 50 times its volume of the hardening agent. As longitudinal rugae of the stomach are usually present it is better to make transections. Imbed, section, etc., as in §38–46.

Stain with haematoxylin, or alum carmine 2 to 3 minutes and fluorescine 15 to 30 seconds. Or stain 5 to 10 minutes with picrocarmine. [*References:* Quain, II, 593; Sat. 388; Str. 370; Fl. 430; St. 178; K. 196; (Ar. m. a. (1885) 174).]

(§ 54) **Section of the ileum.**—The sections should be near the caecum where the villi are comparatively few. Either transections or longisections may be made. If longisections are to be made the ileum should be cut lengthwise and pinned out flat during the hardening and imbedding processes. The dorsal or attached aspect of the terminal part of the ileum usually contains so many Peyer's follicles that the relations of the villi and crypts of Lieberkühn are obscured, hence sections of the ventral or free aspect are to be preferred, or if transections are made they should include the ventral part. Some of the sections should be thick, .04 mm., others should be very thin.

Stain with haematoxylin or alum carmine, 2 to 3 minutes. [*References:* Quain, II, 599; S2. 146; Heitz. 597; Str. 380; K. 201; F. & L. 132].

(§ 55) **Section of liver.**—Harden, imbed, etc., as directed in § 37–46.

Stain with haematoxylin 3 to 5 minutes, or with alum carmine the same time, and then 15 to 30 seconds with fluorescine. [*References:* Quain, II, 626; Heitz. 675; B., 159; Str. 407].

(§ 56) **Section of ovary.**—The greatest care should be taken not to touch the surface of the ovary in the various processes, as there is danger of removing the surface epithelium. Imbed and place in the microtome so that transections shall be made. Some of the sections should be as thin as possible in order to see clearly the primordial ova; others should be thicker, so that the larger ova may be seen. Harden, etc., as in § 36, 39–46.

Stain with carmine or picrocarmine 15 to 20 minutes, or with alum carmine 3 to 5 minutes and fluorescine 15 to 20 seconds. *References:* Quain II, 716; Str. 510; K. 257; Bal. I, 43, II, 614; (Q. j. m. s. (1878) 384, and (1876) 190) K2; (Ar. m. a. I. 162, and XIX 464); S1. 238; Ru. 142; F. & L. 232].

(§ 57) **Section of human skin.**—It is desirable to have sections from two regions,—from the scalp and from the palmar aspect of the hand or the plantar aspect of the foot.

The skin may be hardened as in § 37. The sections are best made by the celloidin method (§ 58). If the paraffin method is used, the greatest care is necessary not to overheat the tissue or the epidermis will become too hard to cut.

Sections from the hand or foot should be vertical to the surface. In making sections of the scalp, the tissue should be arranged, if possible, so that the sections, while vertical to the surface, shall include the entire length of the root of the hair.

Sections may be stained with haematoxylin or with alum carmine 1 to 3 minutes, and then with fluorescine. Paraffine sections may be colored with picro carmine. *References:* R. 876; Quain II, 236; Heitz, 553; K. 274; F. & L. 165; (Ar. m. a. XIII, 205, XVI, 343)].

CELLOIDIN METHOD.

(§ 58) **Celloidin sections.**—The heat required for infiltrating and imbedding with paraffin acts injuriously on some tissues, and some others cannot be so well prepared by the use of paraffin as by the use of celloidin or collodion (a solution of pyroxylin). If it is undesirable to remove the fat from a tissue, both the paraffin and celloidin methods must be avoided and the sections must be made with the freezing microtome (§ 59).

Imbedding in celloidin.—All of the water must be removed from the tissue with 95 per cent. or stronger alcohol; then it should remain from 12 to 24 hours in equal parts of ether and 95 per cent. alcohol. It should then be transferred to a thin solution of celloidin (§ 101, 104) and allowed to remain 12 to 24 hours. It is then placed in thick celloidin (§ 104) for a day, and is finally imbedded as follows: A small amount of thick celloidin is placed in a short test tube, and a cork slightly smaller than the test tube is forced down upon it, after which sufficient celloidin is added to cover the cork for 2-3 mm. After an hour or more the tissue is placed in the test-tube and properly arranged, and finally covered with thick celloidin. The tube is left uncorked until a film forms over the exposed celloidin. The tube is then nearly filled with pure chloroform and corked. The chloroform should be changed two or three times, and in the course of five or six days, or even less, the celloidin will become hard and transparent. The tissue is then ready to be cut. It is usually easy to remove it from the tube after pouring off the chloroform.

Cutting the sections.—If a well microtome is used the imbedded tissue must be wedged in with a cork. A sliding microtome is preferable. The cork on which the tissue is supported is fastened into the holder of the microtome, the knife is made very oblique so that a drawing cut will result. The tissue and knife must be thoroughly

and constantly wet with alcohol of 70 to 80 per cent, and the sections as they are cut should be transferred from the knife to 70 to 80 per cent. alcohol with a quill duster.

Staining and mounting the sections.—The slides to be used for celloidin sections are lightly brushed with collodion or celloidin as directed in (§ 41), the sections are transferred to the collodionized slide and dehydrated by pouring upon them 95 per cent. alcohol with a dropping tube, and then after removing most of the alcohol several drops of equal parts of ether and alcohol are added exactly as for paraffin sections (§ 41). The slide is kept in the air until most of the ether and alcohol have evaporated,—until the section begins to look dull—then the slide bearing the sections may be placed in a jar of 80 per cent. alcohol or several drops may be poured upon the sections with a dropping tube. The sections may now be washed with water, stained, dehydrated, cleared and mounted, exactly as described for paraffin sections (§ 43-46). * † ‡ § *References:* W. 108, Lee., 193; A. s. m. (1884) 209).

FROZEN SECTIONS.

(§ 59) **Sections with a freezing microtome.**—For the study of many objects it is undesirable to subject them to the processes necessary for paraffin or celloidin sections. This is especially true of tissues in which the fat is to be studied. In such cases the sections may be made with a freezing microtome. The freezing microtome also enables one to obtain sections of fresh or hardened tissues in a minimum of time, or when it is desired to treat the sections with silver nitrate or gold chloride.

Cutting the sections.—A piece of tissue of the proper size, either in the fresh state or after hardening, may be placed in the freezing microtome and frozen either with ether vapor, etc., or ice and salt, or ice and 95 per cent. alcohol [S. r. II, 134]. Preferably, however, the tissue is first infiltrated with gum arabic for a day or more and then frozen in the same, or gelatin may be used for the infiltration and also

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* Collodion made from the best gun cotton, that used in photography, is nearly or quite as celloidin.

† The clearing mixture composed of carbolic acid and turpentine (§ 94) answers well for celloidin sections. Chloroform may be used instead [W. 114, Lee, 199].

‡ Tissues imbedded in celloidin may be kept a long time, perhaps indefinitely, in chloroform or 70 to 80 per cent. alcohol. The sections may be kept in a vial of 80 per cent, alcohol until one is ready to stain and mount them.

§ The method here given of fastening the sections to the slide was suggested by H. E. Summers (§ 41): Unless the sections are to be kept in serial order it is not absolutely necessary to fasten them to the slide. If they are to be in series the mass should be trimmed asymmetrically, and the sections as they are cut must be tranferred to numbered watch-glasses. The asymmetrical outline will enable one to get them right side up. See § 60.

for surrounding the object during the freezing and cutting. If gelatin is used, the tissue and gelatin must be kept warm during the infiltration. Alcohol must be removed from the tissue if it is present before infiltrating and freezing in either gum arabic or gelatin.

In cutting the sections the knife should be cooled, and carried directly across the tissue with a rapid movement as for paraffin sections (§ 40).

Mounting, staining, etc.—As the sections are cut they may be transferred to slides, and a drop of glycerin added to each if they are not to be colored. Or if gelatin is used for imbedding, etc., a drop of the desired coloring matter is put upon the sections, and later washed away with a gentle stream of water, and then a drop of glycerin added to each. Seal the preparation as in § 22.

If gum arabic is used for the infiltrating, etc.. and the sections are to be stained, they must be placed for half an hour or so in water to dissolve the gum arabic. They may then be transferred to slides, stained, mounted in glycerin and sealed as directed in § 27. [*References:* Ru. 164; R. 83; W. 76; Sat. 17; S1. 123; (Q. j. m. s. (1884) 163), (S. r. II. 134)].

SERIAL SECTIONS.

(§ 60) Serial sections.—"For the investigation and understanding of embryological and histological facts, only next in importance to the proper hardening of the specimen is the method of serial sectioning and mounting: for the parts may then be compared in the sequence they occupied in the animal or organ, and the most delicate or loosely connected part is preserved *in situ*." For serial sections the object, whatever it may be, is properly hardened, and then dehydrated, infiltrated and imbedded as described in § 38–39; but in order that the most complete and certain knowledge may be obtained from the sections, it is necessary to know with absolute certainty all the aspects of each section; and if the object is an embryo or small animal it is desirable to know the position of the various sections with reference to the ends of the body or definite landmarks. Hence (1) An outline drawing of the embryo or animal should be made before imbedding it. This drawing should be exactly natural size or some definite proportion; as twice natural size, etc. (2) The object should be imbedded so that the sections shall have a definite relation to the *axon* (body axis) and to the three principal planes of the body [W. & G. 33; (A. m. s. (1884) 203); *Wilder*, New York Medical Journal, Aug. 2, 1884, p. 113].

As the tissue is taken from the body it is arranged in a definite manner and pinned to a cork. This arrangement is stated on the label which should accompany the tissue until it is finally mounted. The aspects of a small animal or embryo may of course be determined by simple inspection any time before imbedding. The sections should be made in one of the three principal planes of the body:

(A) *Transections.*—The object is cut in a plane at right angles to the axon (long axis of the body) and the object is so imbedded that the caudal end of the animal or organ is up and the dorsal aspect toward some fixed part of the microtome. Then the sections are placed on the slide with the caudal aspect up and the dorsal aspect toward the top of the slide (away from the label). The right and left of the sections will then correspond to the right and left of the observer.

(B) *Dextro-sinistral longisections.*—The sections are made parallel with the axon (body axis), and the object is imbedded with the dorsal side up and the cephalic extremity in a known position. The sections are placed on the slide so that their cephalic end shall point toward the top of the slide. These sections, if of an animal or embryo, are bi-laterally symmetrical like the preceding, and right and left correspond to the right and left of the observer.

(C) *Dorso-ventral longisections.*—The sections are cut parallel with the axon. The object is imbedded so that the cephalic extremity is known and the left side is up. The sections are arranged on the slide so that the cephalic end shall point toward the left side of the slide, and the dorsal aspect toward the top of the slide.

(D) When ready to make the sections, the distance the microtome screw is out is noted, and when a slide is filled with sections it is again noted. The distance that the screw is raised in cutting the sections on a slide is equal to the total thickness of all the sections on that slide; or in other words, the length of the object cut in making the sections. The amount should be put on the temporary label of the slide and the same should be done for each succeeding slide. In this way one may know, by comparison with the previously made diagram the exact locality from which each slide of sections was obtained.

(E) If an enlarged model is to be made, the sections should be cut of uniform thickness. Born recommends that this thickness be not greater than .04 mm. [Ar. m. a. (1883) p. 584); (M. j. (1876) 578); (A. n. (1884) 446); W. 216].

Cutting the sections.—After the tissue is properly imbedded and placed in the holder of the microtome the imbedding mass is cut away so that only a thin layer surrounds the tissue. This should be trimmed so that it is square or rectangular in outline. The knife is set or held so that its edge is parallel with one side and then it is carried directly and quickly across the tissue. If the imbedding mass is of the right hardness for the temperature, ribbons of indefinite length may be made with a sliding microtome. Short ribbons may also be made, after a little practice, with a well microtome.

Placing the sections on the slide.—The sections may be placed on the collodionized slide (§ 47) singly as they are made, or the ribbon of

sections may be broken into segments of the proper length. In either case the first section should be placed in the upper left hand corner of the slide, and the others should follow as in columns of figures. The sections are fastened to the slide, stained, mounted, etc. as directed in § 42 to 46.

Slides for serial sections.—Unless the object is very small, slides 50 x 75 mm. and rectangular cover-glasses 48 x 58 mm. are recommended. The covers can be more safely spread with the balsam if they are laid flat.

Label for the slides in a series.—This should give the following information. (a) The number of the series. (b) The number of the slide in the series. (c) The date on the label belonging to the animal or organ. (d) The name of the object. (e) The number in the series of the first and last section on the slide. (f) The thickness of all the sections on the slide or preferably the location in the length of the object. (g) The thickness of the individual sections, if a model is to be made from the series. (h) The thickness of the cover-glass:

Series No. 5 Feb. 22, 1886.
Slide No. 1.
Transections of Necturus.
Sections 1 to 50.
Microtome screw raised from 0 to 2 mm.
Sections each .04 mm. thick.
Cover-glass .16 mm. thick.

Serial sections may be made by the celloidin method also. The arrangement of the object in the imbedding mass should be as described above. The mass should be trimmed asymmetrically so that one may get the sections a desired side up on the slide and with the desired aspect toward the top of the slide.*

References.—The literature of section cutting and related matters is very extensive. Original papers or summaries may be found in: W.; Lee.; Sat.; Fl.; Ru.; Sl.; Beh. Fol.; St.; Car.; (J. r. m. s.); (A. n.); (Z. w. m.); (Z. a.); (M. z. s. n.); (A. m. s.); (J. a. p.); (Q. j. m. s.); (Ar. m. a.). *The Medical Student*, 1883.]

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*The perfect sharpening of section knives requires great skill and experience. A large, fine oil stone that has been flattened on a surface plate, with emery, and a strop of fine leather nailed or pasted to a smooth board are desirable. [(Deecke, **A. m. s.**, 1882, p. 275); **Fol.** 129; **W.** 85, and **A. n.**, 1885, p. 831.]

FINE INJECTIONS.

(§ 61) The purpose of a fine injection of the vascular system is to determine the finest vessels in a tissue by the aid of a microscope with the same certainty that coarsely injected vessels may be determined with the unaided eye.

(A) *Specimen.*—Choose a young but nearly mature, lean animal. Kill with chloroform, and leave the animal in the anæsthetic at least half an hour.

(B) Inject before the *rigor mortis* appears.

(C) Inject only the part desired, and tie all anastomosing vessels, and all vessels to other parts.

(D) Inject into the artery of the part, leaving the vein open until nearly pure injecting mass escapes, then tie the vein and continue the injection till the part feels hard and is the color of the injecting mass.

(E) After the injection is finished, if a gelatin mass was used (§ 106), cool the part injected by means of snow, ice or cold water.

Injection of the small intestines of a cat.—Place the cat dorsicumbent on the tray and tie the legs laterad with cords. Open the abdomen by a longitudinal and a transverse incision through the skin and abdominal wall. Isolate the vessels of the intestine by tying (1) a large string, like cotton twine, around the large intestine near the caecum. (2) A similar string should be tied around the duodenum including the pancreas. (3) Tie a string around the superior mesentric vein (W. & G. 355) near the pylorus, and open the vein centrad of the string.

Insert a cannula into the superior mesentric artery near its origin [W. & G. 359]. Fill the cannula with warm water or warm salt solution by means of a dropping tube. Warm the syringe, if a syringe is used, by drawing warm water into it and expelling the water; then fill the syringe with injecting mass (§ 106), which should be at a temperature of 33 to 38° C. Connect the syringe with the cannula, and slowly, but continuously force down the piston. As soon as nearly pure injecting mass escapes from the incision in the vein, tie it, and continue the injection till the intestines feel firm and are of the color of the injecting mass. Then tie the superior mesenteric artery peripherad of the cannula and put snow, ice or cold water on the intestines to cool the injecting mass. When the mass is firm in the large vessels it will not escape from the small ones. It usually takes half an hour or more for the mass to set firmly.

After the mass has set, open the free surface of the ileum, and with scissors cut off some of the villi. Mount them in glycerin and examine with the microscope. If the injection is successful the villi will be quite long and the injected blood-vessels will be comparatively straight. If the villi are short and broad and the vessels are wavy,

satisfactory preparations cannot be obtained. If the villi are not satisfactory in the ileum examine them in various parts of the intestine; for often some parts are satisfactory even if the whole intestine is not.

Injection of the kidney of a cat.—The same animal may be used as for the intestines. Insert the cannula into the renal artary, tie a string loosely around the renal vein and open it centrad of the string. Fill the cannula with water or salt solution with a dropping tube, and then connect the syringe containing the injection mass. Force the piston down gradually, and soon the mass will escape from the vein. Tie the vein and continue the injection till the kidney feels quite firm and is of the color of the mass. Cool it as directed for the intestine.*

(§ 62) *Hardening injected tissues.*—Injected tissues should be hardened one or two days in 50 per cent., two in 75 per cent., and then one or more in 95 per cent. alcohol. Or they may be placed two days in picric acid and alcohol, and then in 95 per cent. alcohol (§36). Injected bones and teeth may be decalcified if desired by the picric acid, or preferably the 2.5 per cent. solution in 95 per cent. alcohol, (§ 97). It is especially necessary to harden injected organs in a cool place.

(§ 63) **Sections of injected kidney.**—These should be made precisely as described for cartilage (§ 34), except that the razor and tissue should be wet with 95 per cent. alcohol. As the sections are cut they should be placed in a dish of 95 per cent. alcohol. The sections should include both cortical and medullary substance.

Mounting the sections.—Place the sections on a slide, clear and mount in balsam (§ 27a).†

(§ 64) **Sections of injected intestine.**—These are best made free-hand by grasping the injected and hardened intestine in the left hand, and with the razor cutting from the periphery toward the center. *The edge of the razor should not go beyond the base of the villi.* A section of the whole intestine may be made in this way by rotating it as it is cut. The sections need not be made very thin, and they cannot be made witth one sweep of the knife. The sections should be mounted in balsam. It is desirable to stain them (see note to § 63).

(§ 65) **Injection of the vessels of a frog's foot.**—Kill the frog by placing it in a jar of water containing chloroform or ether for half an hour. Expose the heart, cut off the apex of the ventricle, and insert

* One can make very satisfactory fine injections with the *injecting jar* described in the note to § 37. If gelatin mass (§ 106) is used the tube extending from the jar to the vessel should be wrapped with woolen cloth to prevent the too rapid cooling of the mass.

† A very transparent, contrasting dye to bring out the tissue in injected sections is desirable. Picric acid is very good when red and fluorescine when blue is used for the injection.

a cannula through the ventricle into the *bulbus aorticus*. Tie a soft string around the bulbus so that it will press upon the cannula. Fill the cannula with water or salt solution with a dropping tube, and inject red mass (§ 108). If the injection is successful the skin of the legs, and the web will become very red, and if the web is held up to the light, the injected vessels may be seen.

(66) **Injection of the lymphatics of the frogs web.**—To inject the lymphatics of the web, a small hole is made through the skin distad of the knee and a cannula filled with blue mass (§ 109) is inserted. The syringe is connected with the cannula and the leg is compressed with the thumb and index just bejond the point of insertion of the cannula. After forcing the piston down slightly the leg and foot are pressed by the thumb and index to force the mass by the valves into the lymphatic capillaries. By alternately forcing down the piston and compressing the foot, one can usually fill the lymphatics in one or more of the interdactylar membranes. If the injection is successful the web will be blue, although the blood vessels are injected with red. By examining the web with a magnifier or the ⅔ths objective one can judge of the success of the injection. Both feet should be injected. When the injection is finished, a string should be tied tightly around the leg beyond the incision for the lymphatic injection and the frog should be cooled with snow, ice or cold water.

(§ 67) **Preparing and mounting the web.**—When a successful injection has been made and the frog cooled, the legs should be cut off near the knee. The toes should be pinned apart on cork so as to moderately stretch the web. Then the foot should be hardened in 50, 75 and 95 per cent. alcohol one day each. The web may then be cut out and mounted in balsam [R. 653].

References for fine injections.—Bi.; Fi.; Si.; St.; W.; Lee; *Robin;* Ru.; R.; Sat.; J. r. m. s.].

METHODS OF PATHOLOGICAL HISTOLOGY.

A thorough knowledge of normal histology is necessary (a) to enable one to determine whether there are any pathological modifications in the structure of a tissue; and (b) the exact nature and extent of those modifications when present.

(§ 68) **The methods of pathological histology** are based on the same principles as those of normal histology: (A) The tissue should be put into the hardening agent as soon as possible after the death of the animal or after its removal from the body in a surgical operation. This is even more necessary in pathological than in normal histology, for there is in many cases a greater tendency to disorganization. (B) The tissue should not be allowed to dry. Vials of the hardening agent should be at hand, and only pieces of moderate size should be

hardened; and plenty of liquid should be used. (C) If possible, scraped or teased preparations of the perfectly fresh tissue should be studied in salt solution or normal fluid to see the appearances in the tissue elements before they are hardened and possibly modified by the hardening agents. Staining by drawing fluorescine under the coverglass by a piece of blotting paper is frequently desirable; and the use of osmic acid to fix the tissue elements is sometimes of advantage (§ 23). In all cases where possible, some healthy tissue should be left with the morbid so that the sections shall include both, and then the transition from the normal to the pathological tissue can be more easily appreciated.

Müller's fluid is suitable for hardening most pathological specimens (§ 37). The imbedding and sectioning should be as described in (§ 38–46), or in many cases it is better to use the celloidin method, as no heat is necessary (§ 58); and if it is desirable or necessary to study the fat of a tissue, the sections should be made with the freezing microtome (§ 59). Sections of perfectly fresh tissue may also be made with the freezing microtome, but the crystals of ice which form in freezing tend to tear the tissue and give it a pathological appearance.

References: The microscopical journals; *Ziegler; Gibbs;* C. & R.; Fol.; *Richardson;* Fi.; B. et G.; Bz.; Buck; *Green;* Heitz.; *Peaslee; Seiler; Tyson; Virchow; Woodhead; Wythe;* (A. p. a.); Mittheilungen aus dem Kaiserlichen Gesundheitsamte; Reports of the U. S. bureau of animal industry].

METHODS OF VEGETABLE HISTOLOGY.

(§ 69) **The methods of Vegetable Histology,** are naturally somewhat similar to those of animal histology as protoplasm, and that which has been formed by protoplasm, "*formed material,*" are investigated in the two cases. The formed material of plants differs greatly from that in animals, and in too many cases far greater attention is paid to it in the study of vegetable histology than to the protoplasm. This is comparable with a too exclusive study of "dry bones" in animals.

In vegetable histology as in animal histology, the tissues should be studied:

(A). Fresh, and alive if possible, (see § 15). Normal conditions are as necessary as for the study of living, animal tissues (§ 7). Protoplasm in plants may be anaesthetized by ether, chloroform, etc., as in animals. [*Bernard,* I, 251]. Free-hand sections and teased preparations of fresh tissues often show the structure well. Osmic acid fixes unicellular organisms and plant cells in a natural condition. It may be applied as directed in § 21, or a drop may be drawn under the coverglass after the object is mounted in water or some normal fluid.

(B). Vegetable tissues should be hardened and cut into sections. Picric acid (§ 97), especially Kleinberg's mixture, is often used. Alcohol of 50 and 75 per cent. each one day, and then 95 per cent. is often used also. The hardened tissues may be sectioned freehand without further treatment, or they may be imbedded, sectioned, etc., by the paraffin method (§ 38-46), or the celloidin method, which seems to be preferable in some ways as heat is unnecessary. The use of celloidin precludes the use of most aniline dyes, however, and they are very largely used in staining vegetable tissues. When-

ever it is necessary or desirable to preserve the chlorophyll, oil or resin in a plant, the freezing method (§ 59) is to be preferred.

(C). Staining vegetable tissues with alum carmine, haematoxylin and fluorescine has the same general advantages as in animal tissues, and the stains may be applied in the same manner. Permanent preparations may be made by mounting in balsam, glycerin or glycerin jelly,—or if the color of the chlorophyll is to be retained a saturated solution of potassium acetate, is recommended by Bower & Vines, p 15.

Excellent directions for work in vegetable histology, and for permanent preparations may be found in: Beh.; *Bower & Vines;* C; Car.; *Goodale;* H. & M.; *Strasburger;* Tr.; (J. r. m. s.) (Z. w. m.) (Q. j. m. s.) (Ar. m. a.) (A. m. m. j.) M. The works of Behrens, Bower and Vines, Goodale, Strasburger, and Trelease are devoted exclusively to vegetable histology and methods for the same.

Permanent preparations of expanded plasmodium, may be made by placing pieces of rotten wood on which masses of plasmodium are found on moistened slides, so that the plasmodium touches the slide. Over the whole should be placed a bell jar, or other cover to prevent drying. In an hour or more the plasmodium will, in many cases, spread out on the moistened slide. The slide should be placed in the picric acid solution (§ 97) for half an hour or longer. The picric acid kills and fixes the extended plasmodium. It may then be rinsed in water and mounted, by covering with a cover-glass well spread with glycerin jelly or it may be dehydrated, cleared and mounted in balsam (§ 27a). Preparations of the common yellow plasmodium prepared and mounted in Canada balsam, as here described, are at present as perfect in color and in every way as when first made five years ago. [*Proc. Amer. Assoc. Adv. Sc.*, 1880, p. 377); (A. m. m. j. (1880) 130)].

THE MICROSCOPE IN JURISPRUDENCE.

(§ 70) While an *entire* human body may be distinguished as such with certainty, histological knowledge is not, in my opinion, sufficiently advanced at the present day to enable one to say *that any microscopic structure is absolutely characteristic of and peculiar to a human being.*

While it is true that no one can say that a given microscopic structure is part of a human being, and not of any other animal, he might say with certainty, that it could not be from some animals in which the given structure is known to be very different. The histological or microscopical expert, in my opinion then, cannot give positive evidence with regard to the exact source of any microscopic structure. The *most* he can do is to say what it may be, and what it cannot be. Even this, unsatisfactory as it may seem, requires a profound knowledge of human and comparative histology, and of the changes that may be produced in the various structures by drying and dampness, by chemical and mechanical means. There is also often required a thorough knowledge of optics, and great manipulative skill. He, who, in the name of Science, allows himself through ignorance or design to become an advocate and not an expounder of the whole truth, so far as it is known, has been well characterized by Woodward as possibly more dangerous to society, than the criminals he is called upon to

convict; and the lawyer who through ignorance or design allows truth and justice to be so betrayed, is no better.

The histological or microscopical expert is called upon most frequently to give evidence concerning the following subjects: (1) Blood or blood stains. (2) Semen or seminal stains. (3) Foetal liquids, excreta or their stains. (4) Contents of the stomach, especially food. (5) Various tissues of the body. (6) Hair, wool, and various textile fibers. (7) Foods; adulteration, or the presence of trichinæ, or larval tape-worms in the same. (8) Adulteration of medicines and other commercial products. (9) Poisons, animal, vegetable and mineral. (10) Handwriting.

In the University Library, or the Anatomical Laboratory may be found the following references to these subjects :*

(1) **Blood and blood stains.**—(a) Normal structure of blood; L. Elsberg, Annals of N. Y. Acad. of Sciences, Vol. I, Nos. 9 and 10. Also in **Heitz**, p. 64; **Fi.** 229; **Ki.** II, 320; **Quain** II, 23; **R.** 178; **Str.** 263; **Todd** I, 404; *Milne-Edwards* I, 49, 83; **Sat.** 34.

(b) *Examination of blood stains.—Richardson*, 284; also Monthly Microscopical Journal of London, 1874 (Reprint). Chemical News, 1875 (No. 132, p. 291); *Taylor's Med. Jurisp.; Wharton and Stillé's medical jurisprudence*, p. 678; Hofmann, 273, 776. The application of photography to the micrometry of blood in criminal cases. Reprinted from the Phila. Med. Times, 1876. **Fi.** 239; **Wormley**, 708; (**A. m. m. j.** III. 6, I. 184); *Stowell's* micr. diagnosis, p. 30; **St.** 82.

(c) *Measurement of blood-corpuscles and precautions necessary in micrometry in general.*—The value of the results vary in proportion to the accuracy of the micrometer and the microscope, and the skill of the observer: **Bi.** 156; **C.** 95, 97, 184, 713; **R.** 31; **Wormley** 701; *Richardson*, 59. Monthly Micr. Journal of London, 1874 and 1875 (Reprints). Woodward, the application of photography to micrometry; and in American Journal of the Medical Sciences, 1875, p. 151. (These papers, and the photographs made by Dr. Woodward of blood and blood stains, should be in the hands of every expert and every lawyer); (Rogers in **A. q. m. j.** 97); (**Q. j. m. s.** (1861 p. 87); *American Journal of Science*, 1872; *Milne-Edwards* I. 83; **Heitz**, 66; International Medical Congress 1876; (**Q. j. m. s.** (1861) 81); *Taylor*, 307; *Wharton & Stillé*, 679; Proc. Zoöl. Soc. of London, 1862, 96, 1875, 474; (**A. m. m. j.** IV 175, V 17, VI 107, 138, 127); (**M.** III, 157); **Buck** I, 584.

(d) *Mistaking spores of plants for blood-corpuscles.*—**Bi.** 156; *Taylor; Richardson*, Monthly Micr. Journal of London, 1874 (Reprint).

(e) *Menstrual blood.*—Dalton, Physiology, 559; *Richardson*, 300; *Littré*, Dict. de med.; *Taylor; Wharton & Stillé*, 677; **Buck**, I, 584.

(f) *Blood crystals.*—Wharton & Stillé, 681; Chemical News (1873) p. 291; (**Fi.** 237; **F2.**; **Quain** II. 27; *Richardson*, 294; *Taylor.*

(g) *Microspectroscopic tests for blood.*—**Bi**, 273; Chemical News, 1873, p. 291; Foster's physiology; Hermann's Physiologie; (**J. a. p.** IV, 119); *Richardson*, 298; (**Q. j. m. s.** (1869) 358); (**P. r. s.** 1866–7, 433; 1863–4, 355); **Buck**, I, 578.

(2) **Semen and Seminal Stains.**—(a) *Normal semen:* **Quain**, II, 697; **Str.** 459. (b) *Seminal Stains:* **B. et G.**, 547, 552, 863; *Rich-*

* These references do not include Bouvier's Law Dictionary, or the Merritt King Law Library, recently obtained by the University.

ardson, 300; *Taylor; Wharton & Stillé*, 452; *Hofmann*, 673; *Hamlin, in* A. m. s., 1883, p. 21, gives the best directions for the treatment of seminal stains.

(3). **Fœtal Liquids, and excreta and their stains.**—Robin et Tardieu, memoir sur l'examen microscopique des taches formées par le méconium et l'enduit fœtal. Paris, London and New York, 1857; B. et G. 575, 581,836; *Taylor; Wharton & Stillé*, 680.

(4) **Contents of the stomach, especially food.**—B. et G. 715; *Stowell*, diagnosis, 35; *Hofmann*, 551; *Taylor;* Wharton & Stillé.

(5) **Various tissues of the body.**—The works of Quain, Stricker, Ranvier, and the journals containing histological structure.

(6) **Hair, Wool and various textile fibers.**—Quain, II; Str.; Todd, II, 478; B. et G., 805; Bulletin of the National Assoc. of Wool Growers, 1875, p. 470; Wurtz, dictionnaire de chemie, II, p. 154, III, 253; *Hofmann*, 291; Fl. 270; Ru. 127; C. 703; B et G. 89; *Stowell*, diagnosis, 115; (A. m. s. 1884, 59–).

(7) **Foods, and their Adulteration.**—*Stowell*, micr. diagnosis, p. 33; (J. r. m. s., 1879, p. 938 and 1881, p. 962); (M. II, 107); Blyth, foods, composition, etc.; (A. m. s., 1883, p. 97); Report, S. M. Babcock, Chemist N. Y. State Agr. Experiment Station, 1885; B. et G., 515. For trichinæ, etc. See *Taylor;* B. et G., 716, 718; Phin: How to detect Trichinae and how to avoid them. Rochester, 1881.

(8) **Adulterations of medicines and other commercial products.**—Hoffmann, Examination of medicinal chemicals, N.Y., 1877; Naquet's legal chemistry. For the adulteration of textile fabrics, see references to (6).

(9.) **Poisons**—*Taylor;* Wharton and Stillé; *Hofmann; Wormley;* B. et G., 718; (A. m. m. j., I, 118).

(10) **Handwriting.**—(A. m. s., 1879, p. 46, 1884, p. 47); (A. j. m. s., IV, 194); (A. m. m. j., I, 48, 124); (A. n., I, 167).

REAGENTS AND THEIR PREPARATION.

Employ only pure materials. Keep all volatile substances (alcohol, ether, xylol, etc.) well covered. Use glass stoppers for the cleaning mixture for glass, nitric and hydrochloric acid. Glass or cork stoppers may be used for the other substances.

(§ 71) **Water.**—The water to be used in microscopial work should be (a) distilled; (b) fresh snow or rain water boiled in an agate or porcelain dish and filtered; (c) soft spring water, boiled in an agate or porcelain dish and filtered. Wherever buildings are heated by steam, distilled water may be easily obtained by tapping the return pipes.

(§ 72) **Cleaning mixture for glass.**—

Water (ordinary water may be used), 2000 cc.
Potassium dichromate, ... 200 grams.
Commercial sulphuric acid (strong),. 200 cc.

Dissolve the potassium dichromate in the water and then add the sulphuric acid. This mixture will remove balsam, sealing mixtures, etc. A prolonged stay in it does not injure glass.

(§ 73) **Normal fluid.**—White of egg 15 cc. Water 200 cc. Mercuric chloride $\frac{1}{20}$th gram. Common salt (na cl) 4 grams. This mixture should be thoroughly shaken in a bottle with bits of glass to mix the albumen with the water. It should then be filtered, and kept in a cool place. The mercuric chloride (*corrosive sublimate*) retards decomposition without being injurious to white blood-corpuscles or ciliated cells.

(§ 74) **Normal salt solution.**—Water 100 cc. Common salt $\frac{6}{10}$ths of a gram.

(§ 75) **Sealing mixture for blood, etc.**—Paraffin 50 grams. Vaseline 50 grams.

(§ 76) **Curara.**—Curara $\frac{3}{10}$ths of a gram. 95 per cent. alcohol 20 cc. Water 20 cc. Grind the curara with the alcohol and water in a mortar. Do not filter. Curara varies in strength. The proportions here given might be too strong in some cases and too weak in others.

(§ 77) **Osmic acid solution.**—Osmic acid 1 gram. Water 100 cc. The water should be distilled or freshly boiled snow or rain water. The bottle should be thoroughly cleaned with the cleaning mixture (§ 72). Put the water into the bottle, break off one end of the sealed tube containing the osmic acid and then, being sure that the tube is clean, drop it into the bottle. It requires several hours for the osmic acid to dissolve. Cover the bottle with black paper or velveteen to exclude light. Osmic acid is very irritating to mucuous membranes, hence care should be taken to keep its fumes from the eyes, nose and mouth.

(§ 78) **Nitric acid 20 per cent.**—Concentrated nitric acid 77 cc. Water 23 cc. A 20 per cent. solution of hydrochloric acid also forms an excellent dissociating agent for muscular fiber-cells and cardiac muscle.

(§ 79) **Acetic acid.**—Glacial acetic acid from 1 to 2 cc. Water 99 or 98 cc.

(§ 80) **Glycerin.**—Pure glycerin. This is often employed for objects that have been hardened. For fresh specimens and those dissociated in 35 per cent. alcohol, glycerin 75 cc, water 25 cc. is used. For objects stained in carmine, 1 cc. of acetic acid is sometimes added to each 100 cc. of glycerin.

(§ 81) **Alum carmine glycerin.**—Glycerin 85 cc. Alum carmine (§ 87) 10 cc. Fluorescine (§ 89) 5 cc.

(§ 82) **Fluorescine glycerin.**—Glycerin 75 cc. Fluorescine 25 cc.

(§ 83) **Glycerin jelly.**—Soak 25 grams best gelatin in water for half an hour or more; pour off the superfluous water and melt the gelatin in a porcelain or agate dish over a water bath. Add 10 cc. of the normal fluid (§ 73) or a little egg albumen and heat the melted gelatin for half an hour or more to clarify it. Pour off the clear portion and add to it 5 grams of chloral hydrate. This clarified galatin is that to be used in making frozen sections with gelatin (§ 59). For glycerin jelly, mix equal parts of the clarified gelatin and glycerin, and shake thoroughly. Keep in a warm place for some time to get rid of air bubbles. "When used, the jelly must be liquified by gentle warmth, and it is useful to warm both the slide and the cover-glass previously to mounting." Care must be taken to avoid air bubbles as they do not disappear as with balsam. Seal as for balsam preparations (§ 27a) after the jelly is cold.

(§ 84) **Shellac.**—Shellac for sealing preparations is prepared by adding shellac (either bleached or that in thin laminæ) to 95 per cent. alcohol in sufficient quantity to make a solution of creamy consistency. This should stand in a warm place for the insoluble parts to settle. The clear part is poured off, and to each 50 cc. of shellac 1 cc. of castor oil is added. This cement may be thinned at any time by adding 95 per cent. alcohol.

(§ 85) **Gold-size.**—This is best bought of dealers in microscopical supplies. It is considered to be one of the best cements for sealing. It hardens slowly. It should not be used for balsam preparations without first using shellac. It is well to add several coats of gold-size to all glycerin preparations sealed with shellac.

(§ 86) **Liquid gelatin.**—Gelatin or clear glue 75 to 100 grams. Commercial acetic acid (No. 8) 100 cc. Water 100 cc. 95 per cent. alcohol 100 cc. Glycerin 15 to 30 cc. Crush the glue and put it into a bottle with the acid, and set in a warm place, and shake occasionally. After three or more days add the other ingredients. This solution is excellent for fastening paper to glass, wood or paper. The brush must be mounted in a quill or wooden handle. For labels, it is best to use linen paper of moderate thickness. This should be coated with the liquid gelatin and allowed to dry. The labels may be cut of any desired size and attached by simply moistening them as in using postage stamps.

(§ 87) **Alum carmine** (*Grenacher's*).—Saturated aqueous solution of alum 100 cc. Best carmine 1 gram. Boil 15 to 20 minutes. Finally add 2–4 grams of chloral hydrate. Sometimes a deposit is left in the tissue. If a small amount of normal fluid or egg albumen is shaken

with the solution and then the bottle be placed where it will be heated to 70–90 C. for a few hours the defect will be remedied. This solution may be diluted with saturated alum solution or with water.

(§ 88) **Haematoxylin.**—Water 150 cc. Alum 5 grams. Haematoxylin crystals dissolved in 95 per cent. alcohol sufficient to give a deep purple color. The solution improves by standing a few days. A fresh solution should be made about every three months. It is very difficult to prepare a satisfactory haematoxylin dye unless crystal of haematoxylin are used.

(§ 89) **Fluorescine.**—Fluorescine $\frac{1}{10}$ of a gram. Water 100 cc. Chloral hydrate 2 grams. A fresh solution should be prepared every few weeks. Eosin $\frac{1}{20}$th gram, water 100 cc., may be used instead of fluorescine.

(§ 90) **Picrocarmine.**—Carmine 2.5 grams. Ammonia 5 to 10 cc. Picric acid, saturated aqueous solution, 100 cc. Chloral hydrate 5 grams. Grind the carmine in water and place it in a tall jar containing 3 to 400 cc. of water. Allow it to remain till the carmine forms a sediment on the bottom. Pour off the liquid and add the ammonia, then add the picric acid, and sufficient water to make 500 cc. Allow this to stand in a warm place (70 to 90 C.) till the liquid is nearly evaporated, then add water to make 500 cc. of solution. Add 10 to 20 cc. of normal fluid (§ 73) and heat to 70 or 80 C. for a few hours. Filter through filter paper and have absorbent cotton crowded into the neck of the funnel. Finally add the chloral hydrate. See W. 42 for Minot's picrocarmine.

(§ 91) **Carmine.**—Carmine 5 grams. Water 250 cc. Ammonia added drop by drop till the carmine is dissolved. Chloral hydrate 5 grams. The carmine is treated as for picrocarmine, and after the ammonia is added the jar is placed in a warm place till the odor of ammonia is scarcely perceptible. It is then placed in a bottle and the chloral added. Gierke recommends the addition of a small amount of ammonium carbonate [*Z.* w. m. I. 75]. Dilute solutions and a long time in the solution is the rule for staining with carmine.

(§ 92) **Silver nitrate.**—Silver nitrate 1 gram. Water 200 cc.

(§ 93) **Canada balsam.**—Canada balsam (one tube) 25 cc. Chloroform 2 cc. Clove oil 2 cc. Canada balsam so prepared clears any cloudiness that may arise in the collodion or celloidin used for fastening sections to the slide. Canada balsam diluted with xylol, turpentine or chloroform is often used for mounting. Balsam thinned with xylol is best for mounting stained microbes, as the tubercle bacillus.

(§ 94) **Clearing mixture.**—Carbolic acid (melted crystals) 20 cc. Turpentine (*oleum terebinthinæ rectificatum*) 30 cc.

(§ 95) **Chromium compounds.**—Those most commonly employed in hardening tissues are: Chromic acid ¼ to ½ per cent. aqueous solutions. Potassium dichromate 1 to 3 per cent. aqueous solutions. Ammonium dichromate 2 to 5 per cent. aqueous solutions. It requires from 5 days to 5 months or more to harden, with a chromium compound.

(§ 96) **Müller's fluid.**—Potassium dichromate 25 grams. Sodium sulphate 10 grams. Water 1000 cc. This agent hardens any tissue fairly well (§ 37).

(§ 97) **Picric acid.**—Picric acid 1.5 grams. Water 50 cc. 95 per cent. alcohol 50 cc. A one per cent. aqueous solution is often employed and a mixture of picric and sulphuric acids is excellent for many vegetable tissues (Kleinenberg's solution: Saturated aqueous solution picric acid 100 cc. Concentrated sulphuric acid 2 cc. After filtering add 300 cc. of water). An alcoholic solution of picric acid is excellent for decalcifying, especially when it is necessary to preserve ciliated epithelium well. A stronger solution than that given above is preferable for this purpose: 95 per cent. alcohol 100 cc. picric acid 2.5 grams is a good decalcifying agent. With it the bones of an adult frog's head were decalcified in 28 days. The cilia and other soft structures were perfectly preserved.

(§ 98) **Alcohol.**—Ethyl alcohol of various percentages. From my own experience, alcohol of a higher percentage than 95 is unnecessary for any of the processes of histology. It is necessary to use plenty of alcohol and to use it but few times for dehydrating tissues. Commercial alcohol has, in many cases, an acid reaction. It may be rendered neutral by adding a small quantity of pure bicarbonate of soda.

(§ 99) **Chloroform.**—Pure anhydrous chloroform should be used. After it has been used for histological purposes it is good to kill animals with.

(§ 100) **Ether.**—Pure sulphuric ether should be used.

(§ 101) **Collodion.**—Gun cotton (that used in photography) 1 gram. 95 per cent. alcohol 50 cc. Ether 50 cc. This should be kept tightly corked and may be diluted by using equal parts of ether and alcohol if it becomes too thick. Only a thin coat should be painted on the slide, and the ether and alcohol added to fasten the sections should be allowed to entirely evaporate, or some staining agents will color the collodion. If ihe mixture of clove oil and collodion (see p. 26) is used for fastening the sections to the slide there is less danger of staining the collodion. If the sections are curled it is also preferable, as they unroll during the expulsion of the clove oil.

(§ 102) **Xylol.**—Pure xylol should be used. It burns with difficulty and hence is much safer than naptha, benzine, or turpentine.

(§ 103) **Paraffin imbedding mass.**—Hardest paraffin obtainable 90 grams. Very soft paraffin 10 grams. These proportions have to be varied according to the temperature.

(§ 104) **Celloidin.**—Two solutions are necessary: (**A**) *Thin celloidin.* Dry celloidin 2 grams. Ether 50 cc. 95 per cent. alcohol 50 cc. (**B**) *Thick celloidin.* Dry celloidin 5 to 6 grams. Ether 50 cc. 95 per cent. alcohol 50 cc. If gun cotton is used instead of celloidin the same proportions will answer. Sometimes the celloidin (§ 58) cannot be shaken out of the test tube after hardening in chloroform. A paper tube with a cork in one end may be used instead of the test-tube and as soon as a film is formed over the top it may be put into a vial of chloroform. The paper would best be removed on the second or third day of immersion. 80 per cent. alcohol may be used for hardening the celloidin instead of chloroform, but it renders the mass opaque and thus makes it impossible to see the object.

(§ 106) **Carmine, gelatin, injecting mass for mammals.**—Dry gelatin 50 grams. Carmine (No. 40) 5 grams. Water 90 cc. Ammonia 10 cc. Acetic acid q. s. Chloral hydrate 5 grams. Soak the gelatin in water until it is soft, then pour off the superfluous water and melt it in an agate or porcelain dish over a water bath. Grind the carmine to a paste with water, and add the ammonia and the water. This should be filtered through filter paper or absorbent cotton, warmed, and added to the warm gelatin. The acetic acid, mixed with an equal volume of water, should be added slowly while constantly stirring the mass, untill the mass smells very slightly of the acid. The mass should be filtered through fine flannel and finally the chloral should be added. The mass so prepared will not deteriorate for a long time, and is ready to use at any time by simply warming it over a water bath.

(§ 107) **Berlin-blue, gelatin, injecting mass for mammals.**—Dry gelatin 50 grams. Saturated aqueous solution of Berlin-blue 150 cc. Chloral hydrate 5 grams. Soak the gelatin in water for an hour or until it is soft, then pour off the superfluous water and melt in an agate or porcelain dish over a water bath. Heat the Berlin-blue to 80 or 90 C. over a water bath and add it to the gelatin. Heat the mixture for 10 minutes or more and stir it occasionally. Filter it through fine flannel and add the chloral. This mass will last a long time without deterioration. It should be kept in a preserving jar and simply requires to be warmed before using. The mass for amphibia (§ 109) answers well for mammals also.*

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* Soluble Berlin-blue is now obtainable at almost any drug store. About 1 cc. of acetic acid should be added to each liter of the alcohol used in preserving specimens injected with Berlin-blue.

(§ 108) **Carmine, gelatin, injecting mass for Amphibia and other cold-blooded animals.**—Dry gelatin 20 grams. Carmine 5 grams. Water 100 cc. Ammonia 10 cc. Acetic acid q. s. Chloral hydrate 5 grams. This should be prepared exactly as directed in § 106. This mass melts at 20 to 25 C. and may be injected if the animal is at a temperature of 15 C. or above. It also answers fairly well for mammals.

(§ 109) **Berlin-blue. gelatin, injecting mass for Amphibia and other cold-blooded animals.**—Dry gelatin 25 grams. Saturated aqueous solution of Berlin-blue 150 cc. Chloral hydrate 5 grams. Prepare as directed in § 107. This mass is fluid at about 25 C. or less, and may be injected if the temperature of the animal is not below 15 C.

References for § 71 to 109: Bl.; Beh.; Car.; C.; Fl.; Fol.; *Gibbs;* H. & P.; Lee.; *Prudden;* R.; *Robin;* Ru.; Sat.; Sl. & 2; *Seiler;* St.; Tr.; W.; (A. m. m. j.); (A. n.); (A. s. m.); (Ar. m. a.); (J. r. m. s.); L.; M.; (M. z. s. n.); (Q. j. m. s.); (S. r.); (S. b. l.); (Z. a.); Z. w. m.)]. Nearly every original paper in histology and embryology introduces new reagents or special modifications of those already in use. A very full discussion of staining agents is given by Gierke [Z. w. m. 1884–5; translation in A. m. m. j. 1885–6].

(§ 110) **Alum cochineal.**—Cochineal 10 grams. Calcined (burnt) alum 10 grams. Chloral hydrate 10 grams. Water 750 cc. Grind the cochineal (which would best be the whole insects), and the alum together, and add to the water. Boil till about 500 cc. remains, then after cooling filter and add the chloral. This dye may be used instead of alum carmine. It is cheaper, easier to prepare, and apparently just as good as alum carmine. [Ar. m. a. 1880, p. 413.]

REFERENCE BOOKS AND PERIODICALS.

The books and periodicals named below in alphabetical order, are in the laboratory or the University library. They pertain wholly or in part to the microscope, microscopical or histological methods, or to histology, or to all of these subjects. For the method of referring to these works and periodicals in the text, see the prefatory note, p. 1.

For current microscopical and histological literature, the Journal of the Royal Microscopical Society, the Index Medicus, the Zoologischer Anzeiger, and the Zeitschrift für wissenschaftliche Mikroscopie taken together furnish nearly a complete record. References to books and papers published in the past may be found in the periodicals just named, in the Index Catalogue of the Surgeon General's library; in the Royal Society's Catalogue of Scientific Papers; in the Zoological Record and in the bibliographical references given in special papers.

BOOKS.

Bal.—Balfour, F. M.—A Treatise on Comparative Embryology. Two vols., illustrated. Pp. 492 + 653. London, 1880. Structure.

Bau.—Bausch, E.—Manipulation of the microscope. Pp. 95, illustrated. Rochester, 1885.

B1.—Beale, L. S.—How to work with the microscope. 5th ed. Pp. 518, illustrated. London, 1880. Structure and methods.

B2.—Beale, L. S.—The Microscope in Medicine. Pp. 539, 500 fig., 4th ed. Philadelphia, 1878.

B. et G.—Beauregard, H. et Gallippe, V.—Guide de l'élève et du praticien pour les travaux pratiques de micrographie, comprenant la technique et les applications du microscope à l'histologie végétale, à la physiologie, à la clinique, à la hygiène et à la médecine légale. Pp. 904, 570 fig. Paris, 1880.

Beh.—Behrens, J. W.—The microscope in botany. A guide for the microscopical investigations of vegetable substances. Translated and edited by Hervey and Ward. Pp. 466, illustrated. Boston, 1885.

Bell, F. J.—Comparative Anatomy and Physiology. Pp. 555, illustrated. London and Phila., 1885. Structure.

Bernard, Cl.—Leçons sur les phénomènes de la vie communs aux animaux et aux végétaux. 2 vols., pp. 968, 50 fig., 4 plates. Paris, 1878–1879. Structure.

Bessey, C. E.—Botany for high schools and colleges. Pp. 611, illustrated. New York, 1880. Structure and methods.

B-S.—Bourdon-Sanderson (editor).—Hand-book for the physiological laboratory. Text, pp. 585; atlas, 123 plates. Phila., 1873. Reprint of the English edition, with slightly different paging. Structure and methods.

B. et J.—Bourgery et Jacob.—Traité complet de l'anatomie de l'homme. Paris, 1844. Vol. 8, Embryology, including Comparative Neurology and Histology. Structure.

Bower and Vines.—A course of practical instruction in botany. Part I, pp. 226. London, 1885.

Brass, A.—Kurzes Lehrbuch der normalen Histologie des Menschen und typischer Thierformen. Illustrated (incomplete). Cassel und Berlin, 1885. Structure.

Buck, A. H. (editor).—A reference hand-book of the medical sciences. Illustrated (incomplete). New York, 1885+. Structure and methods.

Car.—Carnoy, Le Chanoine.—La Biologie Cellulaire; Etude comparée de la cellule dans les deux règnes. Illustrated (incomplete). Paris, 1884. Structure and methods.

C.—Carpenter, W. B.—The microscope and its revelations. 6th ed., pp. 882, illustrated. London and Phila., 1881. Methods and structure.

Cooke, M. C.—One thousand objects for the microscope. Pp. 123. London, no date. 500 figures and brief descriptions of pretty objects for the microscope.

C. and R.—Cornil, V., and Ranvier, L.—A manual of pathological histology, translated from the French, with notes and additions, by E. O. Shakespeare and J. H. C. Simes. Pp. 784, 360 fig. Philadelphia, 1880. Structure, very few methods.

Drysdale, J.—The protoplasmic theory of life. Pp. 288. London, 1874.

Fol, H.—Lehrbuch der vergleichenden mikroskopischen Anatomie, mit Einschluss der vergleichenden Histologie und Histogenie. Illustrated (incomplete). Leipzig, 1884. Methods and structure.

F. and B.—Foster and Balfour.—The Elements of Embryology. Pp. 486, 2d ed., illustrated. London, 1883. Structure and methods.

F and L.—Foster and Langley.—A course of elementary instruction in practical physiology, 4th ed., pp. 276. London and New York, 1880. Methods and structure.

F1.—Frey, H.—The Microscope and Microscopical Technology. Translated and edited by G. R. Cutter. Pp. 624, illustrated. New York, 1880. Methods and structure.

F2.—Frey, H.—Hand-book of the histology and histo-chemistry of man. Translated by Barker. Illustrated. New York, 1875. Structure and chemistry.

Gamgee, A.—A text-book of the physiological chemistry of the animal body. Part I. pp. 487, 63 fig. London and New York, 1880. Structure.

Gibbs, H.—Practical histology and pathology. Pp. 107. London, 1880. Methods.

Goodale, G. L.—Physiological botany. Pp. 499+36, illustrated. New York, 1885. Structure and methods.

Green, T. H.—An introduction to pathology and morbid anatomy, 2d American from the 3d English ed., pp. 316, 111 fig. Philadelphia, 1876.

Haeckel, E.—The natural history of creation, 2 vols., illustrated. New York, 1883. Also original, 1873. Structure and classification.

H. and P.—Harris and Power.—Manual for the physiological laboratory, 2d ed., illustrated, pp. 213. London and New York, 1882. Histology, methods and physiological chemistry.

Heitz.—Heitzmann, C.—Microscopical morphology of the animal body in health and disease. Pp. 849, illustrated. New York, 1883. Structure and methods.

Hoffman, E.—Brouardel, P., translator.—Nouveaux éléments de médecine légale. Pp. 830, illustrated. Paris, 1881.

H.—Hogg, J.—The microscope, its history, construction and application. New edition, illustrated. Pp. 764. London and New York, 1883.

Howes, G. B.—An atlas of practical elementary biology. Pp. 116. London, 1885. Figures, etc., of structures described in Huxley and Martin (q. v.)

H. and M.—Huxley and Martin.—Elementary Biology, 2d revised edition, pp. 279. London and New York, 1876. Methods and structure.

Kingsley, J. S.—The Naturalist's Assistant. Pp. 228, illustrated. Boston, 1882.

K.—Klein, E.—Elements of histology. Pp. 352, illustrated. London and Phila. 1883. Structure.

K. and S.—Klein, E., and Smith, E. N.—Atlas of histology. The illustrations are by Mr. Smith, from the preparations of Dr. Klein. Text by Dr. Klein. Pp. 448, 48 colored quarto plates. London, 1880. Structure.

K1.—Kölliker, A.—Manual of human histology; translated from the German by G. Busk and T. H. Huxley. Two vols., pp. 932, 313 fig. London, 1853–1854. Structure, and methods.

K2.—Kölliker, A.—Entwickelungsgeschichte des Menschen und der höheren Thiere. 2d edition. Pp. 1033. Leipzig, 1879. Structure.

Lee, A. B.—The microtomist's vade-mecum. A hand-book of the methods of microscopic anatomy. Pp. 424. London, 1885.

Lehman, C. G.—Physiological chemistry. 2 vols., pp. 648+ 547; illustrated. Philadelphia, 1855.

Leidy, J.—Fresh-water Rhizopods of North America. Illustrated. Washington, 1879. Methods and descriptions.

Lewis, W. B.—The human brain—histological and coarse methods of research. Pp. 163; illustrated. London, 1882.

L1.—Leydig, F.—Traité d' histologie de l'homme et des animaux. Pp. 629, 270 fig. Paris, 1866. Structure.

L2.—Leydig, F.—Zelle und Gewebe. Neue Beiträge zur Histologie des Thierkörpers. Pp. 219; illustrated. Bonn, 1885. Structure.

Macdonald, J. D.—A guide to the microscopical examination of drinking water. Illustrated. London, 1875. Methods and descriptions.

M'Alpine, D. and A. N.—Biological atlas, a guide to the practical study of plants and animals. Edin. and London, 1880. Figures of many tissues.

Marsh, S.—Section cutting; a practical guide to the preparation and mounting of sections for the microscope, special prominence being given to the subject of animal sections. 2d edition, illustrated, pp. 156. London, 1882.

Marshall, A. M.—The frog, an introduction to anatomy and histology. Pp. 86. London, 1882.

Milne-Edwards, H.—Leçons sur la physiologie et l'anatomie de l'homme et des animaux. 14 vols. Paris, 1857–1880. Structure.

Owen, R.—Comparative anatomy and physiology of vertebrates. Three vols., pp. 2155, 1471 fig. London, 1861–1868. Structure.

Peaslee, E. R.—Human histology in its relations to descriptive anatomy, physiology and pathology. Pp. 616, 434 fig. Philadelphia, 1857.

Phin, J.—Practical hints on the selection and use of the microscope for beginners. 3d edition, illustrated, pp. 231. New York, 1881.

Prudden, T. M.—A manual of practical normal histology. Pp. 265. New York, 1881. Methods and structure.

Quain.—Elements of anatomy, 9th edition. Edited by Allen Thompson, E. A. Schäfer and G. D. Thane. Two vols.. pp. 1694, illustrated. London and New York, 1882. Structure.

R.—Ranvier, L.—Traitè technique d'histologie. Pp. 976, illustrated. Paris, 1875-1882 (incomplete). Structure and methods.

Richardson, J. G.—A hand-book of medical microscopy. Pp. 333, illustrated. Phila., 1871. Methods and descriptions.

Robin, Ch.—Traité du microscope et des injections, 2d ed. Pp. 1101, illustrated. Paris, 1877. Methods and structure.

Ru.—Rutherford, W.—Outlines of practical histology. 2d edition, illustrated, pp. 194. London and Phila., 1876. Methods and structure.

Sachs, J.—Text-book of Botany; 2d English, from the 4th German edition. Pp. 980, illustrated. London, 1882. Structure.

Sat.—Satterthwaite, F. E. (editor).—A manual of histology. Pp. 478, illustrated. New York, 1881. Structure and methods.

S1.—Schäfer, E. A.—A course of practical histology, being an introduction to the use of the microscope. Pp. 304, 40 fig. Philadelphia, 1877. Methods.

S2.—Schäfer, E. A,—The essentials of histology, descriptive and practical for the use of students. Pp. 245, illustrated. London and Philadelphia, 1885.

Sc.—Schwalbe, G.—Lehrbuch der Neurologie. Pp. 739, illustrated. Erlangen, 1881. Structure.

Schwann, T.—Mikroskopische Untersuchungen über die Uebereinstimmung in der Structur und dem Wachsthum der Thiere und Pflanzen. 4 Tafeln. Berlin, 1839. Structure. Cell doctrine. See below.

Schwann, T.—Microscopical researches, also Schleiden's contributions to phytogenesis. Illustrated, pp. 263. English translation by H. Smith. London, 1847. Cell doctrine in plants and animals. See above.

Seiler, C.—Compendium of microscopical technology. A guide to physicians and students in the preparation of histological and pathological specimens. Pp. 130, illustrated. New York, 1881.

St.—Stowell, Chas. H.—The students' manual of histology, for the use of students, practitioners and microscopists. 3d ed. Pp. 368, illustrated. Ann Arbor, 1884. Structure and methods.

Stowell, Chas. H. and L. R.—Microscopical Diagnosis. Illustrated. Detroit, 1882. Structure and methods.

Strasburger, E.—Das botanische Practicum. Anleitung zum. Selbststudium der mikroskopischen Botanik, für Anfänger und Fortgeschrittnere. Pp. 664, illustrated. Structure and methods.

Str.—Stricker, S.—A manual of histology. American translation edited by A. H. Buck. Pp. 1106, illustrated. New York, 1872. Structure and methods.

Suffolk, W. T.—On microscopical manipulation. 2d edition, pp. 227, illustrated. London, 1875.

Taylor, A. S.—A manual of medical jurisprudence, 8th ed. Pp. 937, illustrated. Phila., 1883.

Taylor, A. S.—Principles and practice of medical jurisprudence, 2d ed. Two vols. Pp. 723+672, illustrated. London and Phila., 1873.

Todd, R. E.—Cyclopædia of Anatomy and Physiology, 5 vols., illustrated. London, 1835–1859. Structure.

Tr.—Trelease, Wm. (Ed.)—Poulsen's botanical micro-chemistry, an introduction to the study of vegetable histology. Pp. 118. Boston, 1884. Methods.

Tyson, J.—Cell doctrine; its history and present state, with a copious bibliography of the subject; 2d ed., illustrated. Philadelphia, 1881.

Tyson, J.—A guide to the practical examination of the urine, 4th edition, pp. 196. Phila. 1883.

Virchow, R.—Cellular pathology as based upon physiological and pathological histology. Twenty lectures delivered before the Pathological Institute of Berlin, 1858. Translated from the German by F. Chance; pp. 554, 144 fig. New York, 1866. Structure.

W.—Whitman, C. O.—Methods of research in microscopic anatomy and embryology. Pp. 255, illustrated. Boston, 1885.

W. and G.—*Wilder and Gage.*—Anatomical technology as applied to the domestic cat; an introduction to human, veterinary, and comparative anatomy. 2d edition. Pp. 575, 130 fig., 4 plates. New York and Chicago, 1886.

Wood, J. G.—Common objects for the microscope. Pp. 132. London, no date. Upwards of 400 figures of pretty objects for the microscope, also brief descriptions and directions for preparation.

Woodhead, G. S.—Practical Pathology. Pp. 484, illustrated. Edinburgh, 1883. Structure and methods.

Wormley T. G.—The micro-chemistry of poisons. 2d ed., pp. 742, illustrated. Phila., 1885.

Wythe, J. H.—The microscopist, a manual of microscopy and a compendium of microscopical science, 4th ed., pp. 434, 252 fig. Philadelphia, 1880.

Ziegler, E.—General and special pathological anatomy, translated by D. Macallister: 2 vol., pp. 360+371, illustrated. Methods in appendix separately printed.

Information relating to the *microscope, histology, pathology*, etc., may also be found in the library or laboratory, in: The Encyclopædia Britannica, Appleton's Cyclopædia and the yearly supplements; Johnston's and the Penny Cyclopædia; Ashhurst's International Cyclopædia of Surgery; Quain's dictionary of medicine; Littré's dictionnaire de medécine, etc.; Reynold's, and Flint's systems of medicine; Watt's

Chemical Dictionary ; Wurtz's Dictionnaire de Chemie ; Coat's manual of pathology ; Mittheilungen aus dem Kaiserlichen Gesundheit_ samte, 1884 : Reports of the U. S. bureau of animal industry. The publications of the Smithsonian Institution ; Publications of the museum of comparative zoology.

Human Anatomy : Allen, Gray, Henle, Hoffmann, Sappey, Gegenbaur, etc. *In Physiology :* Carpenter, Dalton, Flint, Foster, Martin, Yeo, Brücke, Hermann, etc. *In Comparative Anatomy :* Huxley, Owen, Gegenbaur, Bronn's Thier-reich, Brass, Wiedersheim. *In Zoology :* Brooks and Claus. *In Physiological, pathological and Medical Chemistry ;* Hoffman's Zoochemie ; Hoppe-Seyler's Physiologische Chemie ; Naquet's legal chemistry ; Neubauer and Vogel's analysis of the Urine ; Ralfe's physiological and pathological chemistry ; Vaughn's handbook of clinical physiology and pathology ; Wheeler's medical chemistry; Blyth, Foods, their composition, analysis, adulteration, etc. *In Geology:* Credner's Elemente der Geologie; Geike's text-book of geology; Zirkel's microscopical petrography; Teall's British petrography.

PERIODICALS.*

A. J. m. s.—The American Journal of microscopy and popular science. New York, 1876–1881. Illustrated. Methods and structure.

A. m. m. j.—The American monthly microscopical Journal. Illustrated. 1880+. Methods, and structure.

A. n.—American naturalist. Illustrated. Salem and Phila. 1867+. Methods and structure.

A. q. m j.—American quarterly microscopical journal, containing the transactions of the New York microscopical society. Illustrated. New York, 1878. Structure and methods.

A. s. m.—American society of microscopists. Proceedings. Illustrated. Methods and structure.

A. s. n.—Annales des sciences naturelles. Illustrated. Paris, 1824+. Structure.

Ar. a. e.—Archiv. für Anatomie und Entwickelungsgeschichte. Illustrated. 1877+ (1795+) Structure.

Ar. b.—Archives de biologie. Illustrated. Paris, 1880+. Structure.

Ar. i. b.—Archives italiennes de biologie. Illustrated. Turin, 1882+. Structure.

Ar. m. a.—Archiv für mikroscopische Anatomie. Illustrated. Bonn, 1865+. Structure and methods.

Ar. p. a.—Archiv für pathologische Anatomie und Physiologie und für klinische Medicin. Berlin, 1847+. Structure.

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*NOTE.—When a periodical is no longer published, the dates of the first and last volumes are given ; but if still being published, the date of the first volume is followed by a plus sign.

Ar. z.—Archives de zoologie expérimentale et generale. Illustrated. Paris, 1883+. Structure.

I. m.—Index Medicus. New York, 1879+. Bibliography, including histology and microscopy.

J. a. p.—Journal of anatomy and physiology. Illustrated. London and Cambridge, 1867+. Structure and methods.

J. d. a. p.—Journal de l'Anatomie et de la physiologie normales et pathologiques de l'homme et des animaux. Illustrated. Paris, 1864+.

J. d. m.—Journal de micrographie. Illustrated. Paris, 1877+. Methods, and structure.

J. n-y. m. s.—Journal of the New York microscopical society. Illustrated. New York, 1885+. Methods and structure.

Jr. a. p.—Jahresberichte über die fortschritte der Anatomie und Physiologie. Leipzig, 1873+. Bibliography and brief summaries.

J. r. m. s.—Journal of the royal microscopical society. Illustrated. London, 1878+. Bibliography of works and papers relating to the microscope, microscopical methods and histology. It also includes a summary of many of the papers.

J. z. n.—Jenaische Zeitschrift für Naturwissenschaft. Illustrated. Leipzig, 1864+.

L.—The Lens, a quarterly journal of microscopy and the allied natural sciences, with the transactions of the state microscopical society of Illinois. Illustrated. Chicago, 1872-1873. Methods and structure.

M.—The microscope. Illustrated. 1881+. Methods and structure.

M. z. s. n.—Mittheilungen aus der zoologischen Station zu Neapel, zugleich ein Repertorium für Mittelmeerkunde. Illustrated. Leipzig, 1878+. Methods and structure.

M.j.—Morphologisches Jahrbuch. Eine Zeitschrift für Anatomie und Entwicklungsgechichte. Illustrated. Leipzig, 1875+.

N.—Nature. Illustrated. London, 1869+. Structure.

P. B. s. n. h.—Proceedings of the Boston society of natural history. Illustrated. Boston, 1841+.

P. r. s.—Proceedings of the royal society. London, 1854.+.

P. T.—Philosophical Transactions of the royal society of London. Illustrated. London, 1665+.

Q. j. m. s.—Quarterly Journal of microscopical science. Illustrated. London, 1853+. Structure and methods.

R . z. s.—Recueil zoologique suisse. Illustrated. Genève, 1884+. Structure.

S.—Science. Illustrated. Cambridge and New York, 1883+. Structure.

S. r.—Science Record. Boston, 1883-4. Methods and structure.

S. b. l.—Studies from the biological laboratory of Johns Hopkins University. Baltimore, 1879+. Illustrated. Structure and methods.

Z. a.—Zoologischer Anzeiger. Leipzig, 1878+. A weekly bibliography of current zoological literature, including animal histology.

Z. b.—Zeitschrift für Biologie. Illustrated. München, 1865+. Structure.

Z. w. m.—Zeitschrift für wissenschaftliche Mikroskopie und für mikroskopische Technik. Illustrated. Braunsch. 1885+. Methods and bibliography of same.

Z. w. z.—Zeitschrift für wissenschaftliche Zoologie. Illustrated. Leipzig, 1849+. Structure.

Besides the list of periodicals just given, the library contains the following in which papers relating to histology and the microscope are sometimes found:—

American academy of arts and sciences, Memoirs and proceedings; American association for the advancement of science, Proceedings; American journal of neurology and psychiatry; American journal of science; American philosophical society, Proceedings; Annals and magazine of natural history; Archiv für die gesammte, Physiologie; Archiv für Psychiatrie und Nervenkrankheiten; Archives de physiologie normale et pathologique; Biologisches Centralblatt; Brain; Comptes Rendus de l'académie des sciences; Journal of Physiology; Journal of Science; Linnæan soc., transactions; London Edin. and Dublin Philos. Mag.; Neurologisches Centralblatt; Philadelphia academy of natural sciences, Journal and proceedings; Popular science monthly; Revue scientifique; Royal Irish academy, Transactions; Royal soc. of Edinburgh, Transactions; Zoological Record; Zoological society of London, Proceedings and transactions. Zeitschrift für Instrumentenkunde. Pringsheim's Jahrbücher für wissenschaftliche Botanik; Just's botanischer Jahresberichte; Bulletin of the Torrey botanical club; Botanical gazette; Botanische Zeitung; Scientific American; Bulletin de l'académie impériale des sciences de St. Petersbourg; Zeitschrift für physiologische Chemie; Archiv für physiologie.

Corrections.

P. 3, 8th line from the top, for "three ways of varying the magnification of," read: three methods of micrometry with.
P. 3, last line, for "I–VIII," read: 49–56.
P. 6, § 8, 6th line, for "floculent matrials," read: flocculent materials. 9th line, same correction.
P. 9, 9th line from the top, for "matrial," read: material.
P. 14, § 23, 3d line, for "moke," read: make; § 24, for "mamalian," read: mammalian.
P. 17, § 27, 20th line, for "thorougness," read: thoroughness; 31st line, for "fluoriscine," read: fluorescine.
P. 18, § 28, 15th line, for "fill," read: fill.
P. 20, § 32, 13th line, for "connula," read: cannula.
P. 25, for "§ 41," read: § 41–42.
P. 27, § 47, 6th line for "fluoriscine," read: fluorescine.
P. 28, § 50, 4th line, for "transactions," read: transections.
P. 31, 2d line of *, for "quite as celloidin," read: quite as good as celloidin; 3d line from the bottom, for "tranferred," read: transferred.
P. 35, 25th line, for "mesentric," read: mesenteric.
P. 36, § 64, 7th line, for "witth," read: with.
P. 38, § 69 (B), 2d line, for "Kleinberg's," read: Kleinenberg's.
P. 40, (c), for "C. 95, 97, 184, 713," read: C. 111, 207, 786.
P. 41, § 71, 1st line, for "microscopial," read: microscopical.
P. 42, § 77, 8th line, for "mucuous," read: mucous.
P. 43, § 83, 6th line, for "galatin," read: gelatin.
P. 45, § 101, 7th line, for "ihe," read: the.