A COURSE

OF

ELEMENTARY

PRACTICAL PHYSIOLOGY.

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BY

M. FOSTER, M.D., F.R.S.,

FELLOW OF, AND PRÆLECTOR IN PHYSIOLOGY IN, TRINITY COLLEGE,
CAMBRIDGE.
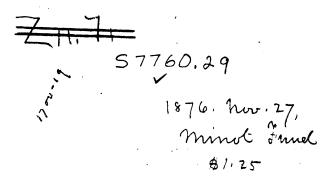
ASSISTED BY

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ST JOHN'S COLLEGE, CAMBRIDGE.

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PREFACE.

EVER since I began teaching Practical Physiology in University College, London, I have been in the habit of distributing among my students a syllabus to guide them in their work. By the introduction of fuller details what was at first a Syllabus has been changed into a Practical Course, which I now venture to publish in the hope that it may be found useful to others.

I fear that its use will be much restricted by the fact that in it Histology and Physiology are closely combined instead of being separated into two or more distinct courses. Although, in practice at least, I stand alone, I believe, in this matter, continued experience makes me more and more convinced that the plan which I adopt is, though troublesome, the safest one. Histological work, unless it be salted with the salt either of physiological or of morphological ideas, is apt to degenerate into a learned trifling of the very worst description; and students are generally only too ready to spend far too much of their time in the fascinating drudgery of cutting sections and mounting stained specimens. In morphological questions the physiologist has but an indirect interest; and details of microscopic structure ought only to occupy his attention in so far as they serve as a basis for physiological deductions. The reader in looking through this little book

will see that in it structure and function go hand in hand. In the case of each tissue or organ, as far as is practicable, the anatomy and histology are first studied, and then without delay the physiology, so that the student may, in learning what is known concerning the action of the part, form an opinion of the relative importance of the structural details.

The various topics are arranged in the order of my lectures, each Lesson containing the practical work corresponding to one or more lectures. In those lectures I first deal with the animal body as a collection of fundamental tissues, each tissue having a conspicuous and characteristic property or characteristic properties, and then consider the ways in which these tissues are arranged in mechanisms, and coordinated into an organic whole. Thus the student is first introduced to the structure and properties of blood, muscle, nervous tissue, and what, for want of a better name, I have called 'indifferent tissues'. Next comes the vascular mechanism, constructed out of the preceding tissues, and carrying on its work by means of their properties. I have found it practically convenient thus to break the series of fundamental tissues by the early introduction of this important mechanism. Returning to the fundamental tissues, the secreting tissues are next considered, under the divisions of the digestive, with which the lymphatics are not unnaturally taken, the respiratory, with which comes the respiratory mechanism, and the urinary, the practical work on the urine being an accompaniment to lectures on the general facts of nutrition. The great nervous mechanism of the body is then attacked, first as regards the special senses and then with reference to the spinal

cord and brain. The Lesson on the larynx is from its very nature disconnected from the others. The tissues of repro‑ duction naturally form the subject of the concluding Lesson.

I have called it an Elementary Course because my class is divided into an Elementary and an Advanced Division, and this work is intended for the former only. I have found it, however, practically convenient to go into some subjects fully in the Elementary Class and to leave certain other sub‑ jects entirely for the advanced class. Thus while, on the one hand, some topics seem to be treated here in a more than elementary manner; others, on the other hand, e.g. the ear and hearing, binocular vision, &c., are entirely absent, being reserved for the advanced class.

I presuppose the student to have gone through a course of Elementary Biology, in which he has worked out the Lessons of Prof. Huxley and Dr Martin. He will thus have acquired a knowledge of the fundamental principles of Histo‑ logy and Physiology, know how to use his microscope and possess a good acquaintance with the Anatomy of the Frog. The dissection of the dog and the rabbit as directed in Lesson I., in which attention is paid only to details of physiological importance, will at once put him in a position for the study of Physiology, strictly so called.

The instructions given are generally complete in them‑ selves; but the student should have access to the Handbook of the Physiological Laboratory, to which (under the ab‑ breviation *Hdb.*) he is frequently referred.

The demonstrations appended to each Lesson illustrate the lecture belonging to the Lesson, and are for the most

part observations or experiments too difficult for the student to carry out for himself. . A list of them is given merely in order that the student may know what he has to see. Nothing therefore is said about the manner of executing them; I may, however, remark that none of them are painful, since they are of such. a nature that anesthetics where necessary can always be used.

The work marked out occupies my class the whole of two terms, *i.e.* from fifteen to eighteen weeks, the lectures being delivered twice weekly, and the practical work being carried on immediately after the lecture as well as on the intermediate days. The plan of work, in fact, closely follows that of Prof. Huxley in his Elementary Biology. It is, of course, not necessary that all the details of each chapter should be worked over by each student. Practical work of this kind is only of value in so far as it suggests or corrects ideas. The student who has mounted an exquisitely thin and beautifully stained section, is only just so much the worse for his pains (as far as physiology is concerned), if he does not understand what the section means. Hence, when the features of some of the fundamental tissues and the general working of the more important mechanisms have been really learnt, and the student has got, by doing things for himself, to know the value of a physiological experiment and the pitfalls that are hidden under carmine and Canada balsam, he may be safely trusted to fill in the details of his study by means of reference to mounted specimens and to mere demonstrations or even to descriptions of experiments. How much practical work is needed by each student must depend on a variety of
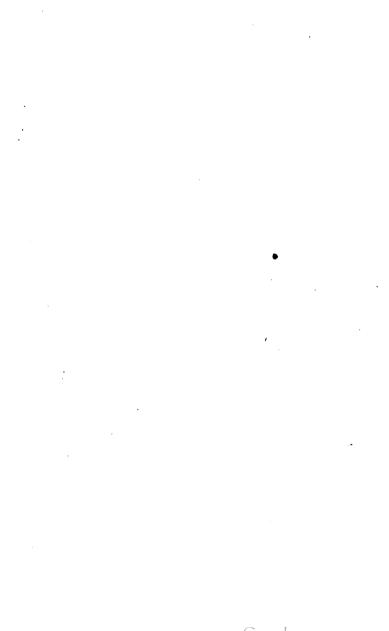
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In no case whatever are the directions given intended to be exhaustive. In the use of reagents, in the details of the microscopical work, and in the various experiments, the counsels given are those which have, on the whole, been found most useful for students in the laboratory. They are not in any way to be considered as forming a practical treatise on Histology or Physiology. That ground is already occupied by the Handbook for the Physiological Laboratory, to which the present little book may be looked upon as an introduction.

So much of the labour of preparing this volume for the press has fallen on my able Demonstrator, Mr J. N. LANGLEY, B.A., of St John's College, that his name naturally appears on the title-page as well as my own. To Prof. Huxley and to Dr H. N. Martin I am indebted for much valuable advice.

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LESSON I.

DISSECTION OF A RABBIT AND OF A DOG.

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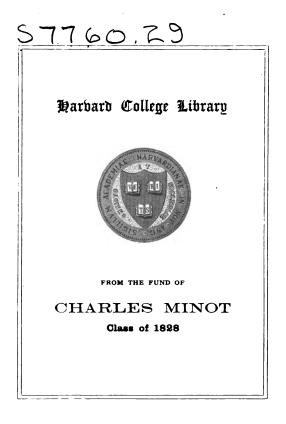
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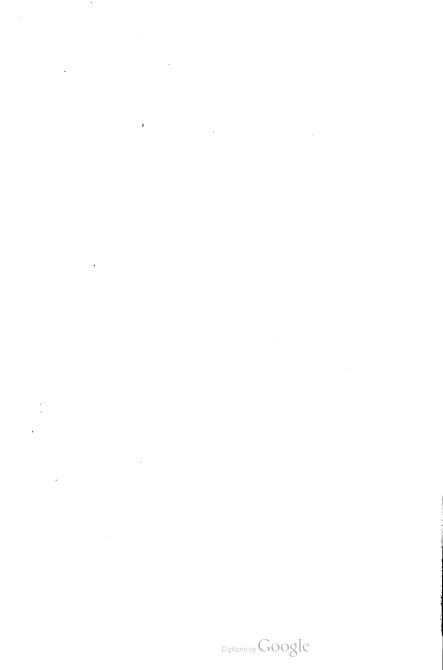
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a. The tendinous aponeuroses of the abdominal muscles forming in the middle line the white *linea alba*.

b. The *externus obliquus abdominis*, a thin muscle, with descending fibres, stretching from the ribs above and from the back to the linea alba and the pubis.

c. The *recti abdominis*, one on either side of the middle line, covered by the tendon of the externus obliquus.

If *b* be carefully reflected, there will be seen underneath:

d. The *internus obliquus abdominis*, with ascending fibres; and underneath the upper part of this

e. The *transversalis abdominis*.

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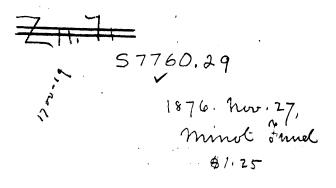FELLOW OF, AND PRÆLECTOR IN PHYSIOLOGY IN, TRINITY COLLEGE,
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L.

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2. Divide the abdominal wall carefully (so as to avoid wounding the intestine) in the middle line from the sternum to the pubis. From the middle of this incision make transverse incisions through the abdominal walls. Hook or pin back the four flaps.

3. Simply turning the parts over without cutting or tearing anything, trace out as far as possible the alimentary canal, making out the **stomach, duodenum, jejunum** passing without any distinct alteration into the **ileum,** the dark sacculated **cæcum** with **appendix vermiformis,** the **large intestine,** (puckered near the cæcum, but in its course becoming smoother and containing balls of fæces,) and lastly the **rectum.** (The student should avoid mistaking the cæcum for the large intestine).

4. Trace out the **mesentery** which supports the intestine ; observe its attachment to the vertebral column behind, its continuity with the lining membrane (peritoneum) of the abdominal cavity, and the manner in which the blood-vessels run in it.

   Observe in the Dog the loose fold of mesentery loaded with fat, hanging from the lower border of the stomach and forming the *great omentum.*

5. Observe the **spleen,** an elongated dark red body lying near the broad end of the stomach to which it is attached by a mesenteric fold (gastro-splenic omentum).

6. Gently stretch out the duodenum and observe in the mesentery belonging to it, the diffuse, pale red **pancreas;** trace the entrance of the pancreatic duct into the duodenum, rather more than a foot below the pylorus.

   In the Dog the pancreatic duct is close to the entrance of the bile-duct (see § 10).

7. Turning the stomach and intestines over to the right side, observe the **dorsal aorta**[1] descending through the diaphragm and giving off first the **cœliac artery,** and next the **superior mesenteric artery.** Just above the latter lies the left **supra-renal body,** and a little above this, lying in the angle between the aorta and superior mesenteric artery, is the transparent **cœliac ganglion,** seen through the peritoneal membrane. Running into this, and crossing the aorta to do so, may be seen the **left splanchnic nerve.** Trace the latter up to the diaphragm. To see these structures more distinctly, the peritoneum and any fat which may be present can be gently removed, care being taken not to injure the splanchnic nerve.

Observe at the same time on the œsophagus the end of the left **pneumogastric nerve,** and trace out the branches of it proceeding to the cœliac ganglion.

8. Then turning the stomach and intestines over to the left side, carefully tear away the connective tissue round the right supra-renal body. Underneath this, or very nearly so, will be seen the right cœliac ganglion, not so large as the left, but having the splanchnic in connection with it. Trace out this latter.

In the Dog the right cœliac ganglion is a little in front of, and nearer the middle line than the supra-renal body. Note the number of pale nerves which are given off, proceeding more especially to the stomach, liver and kidneys.

The left cœliac ganglion will be found in a corresponding position on the other side.

[1] The arteries may be distinguished from the veins by their containing after death little or no blood, and therefore appearing of a bluish white colour, with a central tint of pink, whereas the veins are of a full dark maroon colour.

1—2

9. With the stomach and duodenum still turned to the left side, trace out the union of the mesenteric, splenic, and gastric veins to form the **vena portæ,** and the entrance of that vein into the liver. Observe the **vena cava inferior,** and entrance into it of the **hepatic veins.**

10. Trace out the **hepatic duct,** its union with the cystic duct, and the entrance of the common bile-duct into the duodenum, close to the pylorus.

11. Trace out the division of the cœliac artery into the hepatic, splenic, and gastric arteries: trace out also the main branches of the superior mesenteric artery.

12. Place a ligature round the rectum and another round the œsophagus, and cut through the rectum and œsosophagus a little above the ligatures. Place also two ligatures round the vena cava, one just below the liver, and the other just above that organ, close to the diaphragm. Divide between the ligatures and cut free the liver, taking care not to injure the diaphragm. Cut through the mesentery and remove from the abdomen the alimentary canal and its appendages.

*13. Cut off several small pieces of the liver, about the size of a bean, and place them in Müller's Fluid. Change the fluid next day; and after three weeks transfer to 50 per cent. alcohol, leave two days and transfer to 75 per cent., leave one day and then put in absolute alcohol until required for sections.

In the following sections, when tissues are directed to be placed in Müller's Fluid, potassium or ammonium bichromate, chromic acid, &c.; it is to be understood that they are to be subsequently treated as above with alcohol, unless the contrary is stated.

14. Cut away the mesentery from the alimentary canal, and trace out the latter along its whole length, observing more fully the features mentioned in § 3, and noting in addition the white patches (Peyer's patches) on the free surface of the ileum and the spotted appearance of the vermiform appendix due to lymph-follicles.

Note in the Dog, the shorter length of the intestine, the smaller cæcum, and the less difference between the large and small intestine.

*15. In the Dog (if dissected within 24 hours of its death)

   a. Cut some of the pancreas into small bits the size of peas, and place in absolute alcohol.

   b. Mince the remainder, remove as much fat as possible, dry with blotting-paper, and cover with glycerine.

*16. Lay open the stomach, throw away the contents, and wash the surface once with normal saline solution.

   a. Cut out small pieces of the middle region (one inch square) and place in Müller's Fluid.

   b. Place similar pieces at once into absolute alcohol.

   c. Place in alcohol, or Müller's Fluid, one or two small pieces, taken from the pyloric end.

   d. From a piece of the middle region of the stomach remove the mucous membrane from the muscular coat; mince the former finely; dry it somewhat by pressing it between blotting-paper, and cover it with strong glycerine in a small flask or bottle.

17. Wash out the duodenum, observe the openings of the biliary and pancreatic ducts, and carefully pass a bristle through them into the ducts.

18. Lay open a portion of the jejunum, wash it, and examine the villi with a lens.

*19. Lay open another portion, about 2 inches long, wash it once with normal salt solution, divide it into half a dozen pieces. Place some in chromic acid, ·5 per cent., and others in Müller's Fluid. Lay open several inches of the ileum by an incision carried along the mesenteric attachment. Cut out the Peyer's patches, and place them in Müller's Fluid.

20. Take a piece of the large intestine, cut it open and wash, look at it with lens; there are no villi.

*21. Place two or three small pieces of the spleen in ammon. bichrom. 5 per cent.

22. Remove with a moist sponge any blood in the abdominal cavity; observe the mesenteric glands.

23. Note the position of the supra-renal bodies.

24. Note the position of the **kidneys,** the left being much nearer the pelvis than the right; trace out the renal artery and vein. Observe the **ureter,** a pale semi-transparent duct passing downwards from the kidney over the muscles of the back, towards the middle line; trace it down to its end in the urinary bladder.

25. If a female, observe the **uterus,** with its two cornua prolonged into the Fallopian tubes. Near the end of each Fallopian tube will be seen a small, somewhat oval, spotted body, the **ovary.** If a male, observe the **testis** in its serous pouch, the **epididymis,** and the **vas deferens;** trace the vasa deferentia to their entrance into the **urethra.**

26.   Divide the symphysis pubis, stretch the two halves apart, and with the bone forceps cut away as much bone on each side as may be necessary. Observe the prostate, surrounding the first part of the urethra. Lay open the **bladder,** observe its neck, ending in the urethra, and the entrance at the base of the two ureters.   Pass a probe down each ureter into the bladder.

*27.   Divide one kidney longitudinally, and observe the cortex, medulla, and pelvis. Divide one half into four or five pieces, each containing cortex and medulla, and treat some with ammon. bichrom.; the rest with absolute alcohol.

*28.   *a.*   Make two or three incisions into the ovary, and treat it with chromic acid ·5 per cent.

       b.   Cut off two or three pieces of testis, and one or two of the epididymis, not larger than a pea, and treat with Müller's Fluid.

*29.   Cut off one or two pieces of bladder, and treat with Müller's Fluid.

B. 1.   Make a median incision over the skull from the nose to behind the level of the ears.   Reflect the skin on each side.   Cut away the attachment of the muscles of the neck to the occiput until the occipito-atlantoidean membrane between the occiput and the atlas is laid bare.   Carefully divide this with scissors and observe the medulla oblongata.

With a trephine saw through the roof of the skull in its broadest part, a little behind the orbits, working

very carefully when the bone is nearly sawn through. With an elevator raise the circular piece of bone and remove it. Then with the bone-forceps cut away piecemeal the rest of the roof of the skull.

Observe the smooth **cerebral hemispheres,** somewhat triangular in form; in front of these and separated from them by a constriction, the elongated **olfactory lobes.** Behind the cerebral hemispheres lies the laminated **cerebellum** (the outlying lateral portions of which are lodged in pits in the skull), and behind this again is seen the **medulla oblongata** with the fourth ventricle. On the surface of the cerebrum may be seen the dura mater and pia mater, but these are better seen in the dog.

2.   In the Dog observe

    a.   The *dura mater,* a thick and very resistant membrane; its prolongation between the cerebral hemispheres as the *falx cerebri* and between the cerebellum and cerebrum as the *tentorium.*

    b.   The *pia mater,* underneath (*a*), and immediately covering the brain; note the blood vessels running in it.

    c.   The relative sizes of cerebrum and cerebellum.

    d.   The general appearance of the cerebral convolutions.

3.   With a scalpel divide the front of the cerebral hemispheres from the olfactory lobes. Lift up with the handle of a scalpel the extreme front of the cerebrum, and turning it backwards bring into view the optic nerves. Cut these through with a sharp pair of scissors close to the skull. Still turning the brain back cut through successively all the other cranial nerves. A little behind the optic nerve is the small but evident third nerve (motor oculi), close behind

this the considerably smaller fourth nerve (trochlear), farther back in the hollow behind the attachment of the tentorium lies the thick fifth nerve, to the median side of which the small sixth (abducens) is fairly conspicuous. A little behind and to the outside of the fifth, in the hard petrous bone are seen together the seventh (facial) and eighth (auditory). Some distance back and nearer the middle line come the ninth (glossopharyngeal), tenth (pneumogastric), and the small eleventh (spinal accessory). Lastly, still farther back is the twelfth (hypoglossal). Finally, cut through the spinal cord below the medulla oblongata, and remove the brain entirely.

The outlying lateral portions of the cerebellum will probably be left in the skull. Do not injure the skull in attempting to get them out.

4. Placing the brain bottom upwards observe the superficial origin of the above cranial nerves. (These will be studied more carefully in a subsequent lesson.) Place the brain in spirit.

5. In the Dog repeat § 3 and 4, observing in addition the basilar artery uniting with the internal carotid arteries to form the circle of Willis, which will be seen more distinctly in the dog than in the rabbit.

*6. Remove one eye, puncture the cornea, make a slight incision in the thin part of the sclerotic behind the cornea and place it in strong spirit.

C. 1. Examine the **diaphragm,** marking well its shape; observe the central tendon and the radiating muscular

bundles, their attachment to the chest-walls; the orifices of the œsophagus, aorta, and vena cava.

2. Make similar observations on the Dog, where the diaphragmatic muscles are much more fully developed.

3. Through the transparent tendon of the diaphragm the lungs are visible in close contact with it. Puncture the tendon, and observe the collapse of the lungs upon the entrance of air into the pleural cavity.

In the Dog

4. Observe the *pectoralis muscle* consisting of the *sternal and costal pectoral*, the former partly overlying the latter. Cut through the sternal pectoral in its middle and reflect the two ends. Observe its attachment to the upper part of the sternum and to the humerus. Observe the attachment of the costal pectoral along the greater part of the sternum below the sternal pectoral.

5. Observe the costal cartilages and their connection with the ribs and sternum.

6. Observe the *serratus magnus* proceeding from the whole length of the internal border of the scapula to the first seven ribs. Cut this through the middle.

7. Note the course of the fibres of one of the *external intercostals* about an inch from the end of the bony rib. Trace it forward to about the juncture of the rib and cartilage where it ends in a free border and the fibres of the *internal intercostal* pass beneath it. Beginning at this free border remove the external intercostal, laying bare the internal intercostal; trace also the intercartilaginous part of the latter.

8. Turning the animal on its side observe the *serratus anticus*, arising from a single flat tendon lost amongst

the muscles of the neck and dividing into bundles
going from the 4th rib to the 10th inclusive.

9. Dissect away the conspicuous longitudinal muscles
covering the vertebral ends of the ribs, and observe
the *levatores costarum* arising from the transverse
processes of the dorsal vertebræ and proceeding to
the ribs below.

10. Cut through the costal cartilages at their junction
with the ribs, beginning at the second ; divide the
sternum between the 1st and 2nd ribs, and remove the
sternum with the cartilage. While removing it ob-
serve the **mediastinum** separating the two pleural
cavities.

*11. Place a small piece of costal cartilage in Müller's fluid.

12. Observe the **pericardium,** lay it open.

13. Observe the **vena cava inferior**—and, on each side
of the mediastinum the **phrenic nerve** distributed
to the muscles of the diaphragm. Trace the phrenic
nerve up until it is lost in the tissue at the top of the
chest. Avoid cutting it.

14. Prolong the median skin incision to the chin and re-
flect the skin as far as possible. Observe on each side
the external jugular vein dividing into two branches ;
avoid wounding it.

15. Cut through in the middle line the thin superficial
muscle (platysma) ; draw it to one side, clearing away
the connective tissue. Lying on either side of the
muscles immediately surrounding the trachea will be
seen the sterno-mastoid muscle diverging from the
lower part of the neck. Separate this and draw it to

one side ; there will be seen the **carotid artery,** and running along the outer side of it the **pneumogastric nerve.** Free in one place the carotid, and lift it up with forceps. In the underlying connective tissue will be seen two nerves more or less closely united ; the larger is the **sympathetic,** the smaller the superior cardiac (**depressor**).

16. Clear away the connective tissue from the artery. Passing *over* it at the level of the larynx will be seen the descendens noni. Cut this through and remove it entirely. Passing *underneath* the carotid nearly at the same level is the **superior laryngeal** branch of the pneumogastric. Trace this *with especial care ;* soon after it leaves the pneumogastric it will be seen to give off a small nerve, the depressor. Follow this down the neck, separating it from the sympathetic. Sometimes the depressor receives a branch direct from the pneumogastric. Occasionally this is its sole origin.

17. Remove the first rib and the remains of the sternum, avoiding any injury to the tissues below. Observe the **thymus,** a fatty looking body covering the roots of the great vessels. It may be torn away.
Trace out on each side the junction of the jugular and subclavian veins to form respectively the **right** and **left venae cavae superiores.**

18. Observe the right vena cava superior passing straight down to join the right auricle; the left vena cava superior passing obliquely downwards underneath the left auricle to join the right auricle; and the inferior vena cava passing upwards from the diaphragm to join the right auricle.

19. Trace up the **phrenic** nerve. It makes its way out of the thorax by the side of the superior vena cava, and then passes beneath it. Place a double ligature round the vein and divide between the ligatures. Follow up the phrenic to its origin from the 4th and 5th (and also from the 6th and 7th) cervical nerves.

20. Trace out the arch of the aorta by clearing away the tissue from its upper surface. Take care not to injure the pneumogastric nerves (see next section). Observe on the right side the **innominate artery,** which gives off first the **left carotid,** and then divides into the **right subclavian** and **right carotid;** on the left side the **left sub-clavian.**

21. Trace both pneumogastric nerves downwards, observing the **recurrent laryngeal** branches passing on the right side round the sub-clavian artery, and on the left round the aorta. Place a double ligature round the innominate artery and divide between the ligatures. Trace the recurrent laryngeal nerves along the back of the trachea to the larynx. Pursue the main pneumogastric trunks on the œsophagus to the point where they were seen in A, § 6.

22. Trace the sympathetic nerve downwards to the **inferior cervical** ganglion lying a little above the sub-clavian artery, and close to the vertebral artery; follow it thence to the **first thoracic ganglion.**

Observe the branches going from these ganglia to the heart. Observe also the depressor nerve passing to the heart.

From the first thoracic ganglion trace down the thoracic sympathetic lying on the heads of the ribs

with the ganglia (twelve in all) and the **rami communicantes.**

Trace out the **splanchnic** on one side; it will be found to separate from the sympathetic at the 8th, 9th, or 10th thoracic ganglion. At first sight it appears to be the continuation of the sympathetic instead of a branch of it; since the sympathetic at its lower part becomes more transparent, and running in a groove between two muscles, is rather easily overlooked.

The splanchnic receives branches from each of the thoracic sympathetic ganglia below its origin.

23. Cut out the heart with the lungs attached and trace the **pulmonary arteries and veins.**

The heart may here be dissected by help of the directions given for the dissection of a sheep's heart in Lesson XI., or its dissection may be omitted.

24. Having reflected on either side the skin of the neck of the Dog, and cleared away the fascia of connective tissue, observe the muscles underneath.

a. The *sterno-hyoid* one on either side of the median line.

b. The *sterno-thyroid* lying immediately outside (*a*) for the greater part of its length.

c. The *thyro-hyoid*, a small muscle of the larynx external to (*a*) in the upper part of its course.

d. The *sterno-mastoid* external to (*b*) and covering it near the hyoid bone, thence it proceeds outwards, and disappears under a white oval mass, the submaxillary gland.

25. Carefully separate the sterno-mastoid from the sterno-thyroid ; the sympathetic-pneumogastric trunk **and** the carotid artery will come into view.

26. Observe the following points in which the Dog differs from the Rabbit:

    a. There is but *one* superior vena cava formed by the junction of the *two innominate veins.*

    β. There is in the neck no separate nerve corresponding to the depressor in the rabbit.

    γ. The sympathetic is intimately united with the pneumogastric in the greater part of its course. Low down in the neck it separates from the pneumogastric, though still running in the same sheath with it, until it reaches the inferior cervical ganglion. Issuing from this it diverges from the pneumogastric, going to the large rod-shaped first dorsal ganglion.

D. 1. Make an incision over the knee, and remove the skin for some distance above and below it. Observe the **fascia** covering the muscles of the thigh and leg, the fibrous **capsule** of the knee-joint, and the patella.

2. Remove the fascia, dissect out the lower part of some of the muscles of the thigh, cutting them across in the middle, and observe the attachment, direct or by tendon, to the bones, and the continuation in some cases (e. g. *musc. sartorius*, the thin broad muscle running obliquely across the thigh) of their tendons into the capsule of the knee-joint.

3. Observe the **lateral ligaments** or fibrous strengthenings of the capsule.

4. Move the joint until the edges of the femur and of the tibia are felt, and make a transverse incision through the capsule between the two bones. Observe the **synovial fluid.**

5. Bend the knee so as to bring the **crucial ligaments** into view and divide them; divide also the lateral ligaments, and expose completely the ends of the bones.

6. Examine the extent of the synovial sac. Observe the articular ends of the bones, their cartilaginous coverings, and their form. Observe the interarticular cartilages.

7. Remove the fascia, and clean any one of the muscles of the leg.
    Observe its attachment to the tibia (or fibula) above, notice the fascicular construction of the muscle; the entrance of nerves and blood vessels. Observe how the muscular bundles end below in the tendon.

8. Trace the tendon down into the foot. Observe its synovial sheath.

E. The Student should have a rabbit's and a dog's skull before him, and make out the several openings by which the nerves spoken of below issue from the skull.

1. Sever the neck at its lower part from the trunk, and reflect the skin from the side of the head. Place the head on one side. At the base of the ear will be seen the small parotid gland. From its anterior border issues the **facial** (7th) nerve, branching almost immediately; these branches may be traced to certain facial muscles.

2. Carefully pushing upwards the facial nerve close to the parotid gland, the small pale duct of Stenon will be seen. Satisfy yourself that it is a duct, by gently pushing a bristle down it. Its course need not be traced.
    The duct is seen much more readily in the dog.

3. Dissecting away the parotid gland, trace the facial
    nerve to its exit from the skull by the stylo-mastoid
    foramen; observing the branches going to the muscles
    of the ear. Whilst still in the skull the facial gives
    off the chorda tympani. See § 13.

4. Behind the parotid gland will be seen a nerve running
    from the under surface of the sterno-mastoid muscle,
    dividing into two branches, and passing up the
    ear. This is the **great auricular,** which arises from
    the 3rd cervical nerve, and is the main sensory nerve
    for the ear. Trace as far as possible its course in the
    ear.

5. Place the head resting on the lower jaw, and clear
    away the skin from the base of the ear; a somewhat
    flat piece of cartilage will come into view. In the
    normal animal this can be felt by passing the finger
    from the occipital spine outwards towards the ear.
    Through this cartilage runs another sensory nerve
    of the ear, the **posterior auricular.** Carefully clear
    away the connective tissue on either side of the
    cartilage, and trace the nerve forwards into the ear.
    Trace it also backwards along the neck, to its origin
    from the 2nd cervical nerve.

6. Now follow up the course of the **pneumogastric** from
    the place where it was left in C. § 16. A little
    above the superior laryngeal branch will be seen the
    pharyngeal nerve. Still a little higher up, the trunk
    presents a fusiform enlargement, the **ganglion** of
    the **trunk.** Note the large nerve (**hypoglossal**) run-
    ning across the pneumogastric a little below the
    ganglion. Observe at the same time the sympathetic
    nerve passing into the **superior cervical ganglion.**

L.   2

In the dog the united sympathetic-pneumogastric trunk divides at this point into the sympathetic with its ganglion, and the pneumogastric with its ganglion.

7. In the dog, in which the skin has been reflected, the outline of a muscle attached to the inner part of the extremity of the lower jaw will be seen, this is the digastric. Clear away as much as possible the connective tissue surrounding it, carefully cut it through, and turn back the cut ends; the *submaxillary duct* will be seen running from the submaxillary gland, and passing underneath the mylohyoid muscle.

Cut through the middle of the latter, parallel to the jaw, taking care not to injure the tissues below ; reflect the cut ends. In the fascia below the muscle will be seen the continuation of the submaxillary duct. Crossing the duct not far from the free lower border of the mylohyoid muscle runs a nerve, the *lingual* or gustatory, a branch of the 5th. It is seen coming from underneath the jaw, and passing on to the tongue. Just before it crosses the duct it gives off a small branch, *chorda tympani*, which turning back runs to the duct, and thence to the gland. In the triangle formed by lingual, chorda, and duct, lies the small submaxillary ganglion.

In the middle line is seen the hypoglossal passing to the muscles of the tongue.

8. Trace the chorda tympani back towards the gland. Trace the lingual towards the tongue, it will be found to be mainly distributed to the tip of the tongue.

9. Trace the submaxillary duct from the gland on towards the tongue. Lying on it in its course is seen the small lobulated *sublingual gland,* and issuing from the gland, the sublingual duct. The two ducts (the sublingual being the smallest) run together.

10. In the rabbit when the skin and underlying fascia
    have been reflected, there will be seen between the
    angles of the jaw the tolerably compact submaxillary
    glands meeting one another in the middle line. Trace
    forward the duct and make out as in the dog the
    lingual and hypoglossal nerves. The chorda tympani,
    being small, will not be easily seen.

11. Partly saw through the symphysis menti, then use a
    lever and force the rami asunder, and in the following
    dissection cut through or remove any muscles necessary.

12. Trace the lingual backwards. It will be found to join
    the inferior dentary (a large nerve entering into the
    lower jaw), to constitute, with other branches, the
    inferior maxillary nerve. Trace this back to the
    front edge of the tympanic bulla.

13. The lingual, almost immediately after its leaving the
    inferior maxillary, receives a small nerve, the chorda
    tympani. Carefully trace the latter backwards; it
    will be found to make its exit from the skull by
    the Glaserian fissure, near and rather to the outside
    of the tympanic bulla.

    > If this is not seen in the rabbit, it should be traced out
    > in the dog.

14. Trace the hypoglossal nerve forwards from its point
    of crossing the pneumogastric. It will be found to
    terminate in the muscles of the tongue.

15. Trace up the pneumogastric beyond its ganglion, to
    near its exit from the skull by the foramen jugulare;
    (observe the foramen on the prepared skull).
    Note, passing from the skull with the pneumogastric,
    the small **spinal accessory** nerve behind and the
    glosso-pharyngeal in front.

2—2

16. Trace the glosso-pharyngeal forwards to the tongue
and pharynx. It runs somewhat parallel to, but at a
higher level than, the hypoglossal, and may be traced
to the hinder part and to the sides of the tongue.

17. Cut through the above three nerves, at some distance
from the skull, break away with small bone forceps
the tympanic bulla, and trace back more thoroughly
the exit from the skull of these nerves and of the
hypoglossal. The latter issues through the condyloid
foramen, which is separated by a distinct width of
bone from the foramen jugulare, through which the
other three issue.

18. Saw through the base of the skull and the face, from
the occiput to the nose, nearly in the median line,
but leave the nasal septum entire on one half. Divide
carefully with a scalpel the soft palate and the roof
and back of pharynx.
Observe the cavity of the nose, the anterior and pos-
terior nares, the turbinate bones, and the Schneiderian
membrane.

19. Looking down into the pharynx, observe the **epiglot-
tis** at the root of the tongue, the glottis of the larynx
and the vocal cords.

Look at the side of the pharynx for the opening of the
Eustachian tube, pass a probe up it into the tympanic
cavity. Pass another probe down the meatus exter-
nus and, rupturing the membrana tympani, make
sure that the first probe has entered into the tym-
panic cavity.

20. Remove the larynx. Observe the position and rela-
tions of the hyoid bone. Divide the larynx by a me-

dian incision in front, observe the vocal cords, the arytenoid, cricoid and thyroid cartilages.

In the Dog put the larynx in weak spirit, for dissection at a later period.

21. With a pair of bone forceps remove piecemeal the roof of the orbit which still contains the eye, leaving a broad ring of bone round the optic nerve. Imbedded in the fat will be found the muscles of the eyeball. Trace out the origin and insertion of the four *recti* and two *obliqui*.

22. Immediately below, and in front of the eye, the **superior maxillary nerve** will be found issuing from a foramen in the superior maxillary bone, to supply the skin of the face, &c., with sensory fibres. Cutting away the bone with a small pair of bone forceps, trace this nerve back along the floor of the orbit.

Trace at the same time the inferior maxillary nerve.

23. In the upper part of the intact orbit of the Dog will be found a nerve, the *ophthalmic nerve*. It passes from the front of the orbit to the forehead.

24. These three, the ophthalmic, the superior and the inferior maxillary nerves, when traced back, will be found to unite into one large nerve, the fifth or **trigeminal.** Observe on the nerve at the junction of the three branches, the swelling of the **Gasserian ganglion.** Observe also that the nerve in leaving the brain has two roots, a small and a large, that the small root passes beside the ganglion on the large root, without entering into it, and that the fibres of the small root are beyond the ganglion, almost entirely confined to the third or inferior maxillary branch.

LESSON II.

STRUCTURE OF BLOOD.

A. BLOOD OF FROG OR NEWT.

1. Place a drop of blood on a glass slide and close to it place a drop of normal saline solution, but so that they do not run into one another. Carefully cover with a cover-slip, avoiding air-bubbles. At the line of junction of the two drops the corpuscles are few, and can there be best examined for special characters. Look at the slide first with the naked eye, then with objective no. 3 or 4, and finally with objective no. 7 or 8.

2. Examine the **red corpuscles,** and observe that

   a. Each red corpuscle is a flattened oval, of some breadth when viewed flat, narrow when seen in profile.

   b. Seen singly, each corpuscle appears to be of a pale yellow colour, the tint being equally diffused throughout its substance. Where several corpuscles lie one over the other, they together appear red.

   c. In a carefully prepared specimen, many of the corpuscles appear homogeneous; in others there is visible, owing to changes which have taken place, a central slightly darker spot, the nucleus.

d.   There is a very considerable uniformity in the size of the red corpuscles. Measure the long and short axes of two or three. The simplest mode of measuring objects under the microscope is as follows :

Place a drawing-pad in a firm position close to and on a level with the stage of the microscope. Looking through the tube of the microscope with one eye, keep the other open also. An image of the field of the microscope with the corpuscles will appear on the drawing-pad; and the outline of a corpuscle may, with a little practice, be traced.

Substitute for the object the stage micrometer, marked with lines, which are small fractions of an inch or of a millimetre apart. Trace these in the same way on a piece of cardboard, taking care that the cardboard is at exactly the same level as the drawing-pad was previously. The cardboard can be kept as a scale, and tracings of any object *drawn at the same level*, with the same magnifying power, may be directly measured by it. Of course for each objective, and each ocular, and for each position of the draw-tube, a separate scale is necessary.

With a camera lucida the same mode of measurement is much easier.

In this and succeeding lessons the student is recommended to measure all the more important structures.

3.   Bring a drop of dilute acetic acid (1. p. c.) on the glass slide, so that it just touches one edge of the cover-slip. Be careful that the acid does not flow *over* the cover-

slip. Touching the opposite edge, place on the slide a small piece of blotting paper, so as to absorb the fluid under the cover-slip on that side. There will then be a current from the drop of acetic acid to the blotting paper. It is best to arrange that the current is mainly along the line of junction of the blood and saline drop. Observe the changes in the red corpuscles, looking first at those which the acid has just begun to attack, and then at those more fully acted upon. Note that

a. The corpuscles become more egg-shaped, swelling up from the *absorption of fluid*. Some after a time become spherical.

b. The nucleus becomes more and more distinctly visible, and as it does so becomes granular and irregularly rod-shaped.

c. The colouring matter is either concentrated around the nucleus, or disappears altogether.

d. The outline of the corpuscle is seen as a faint oval line at some distance from the nucleus. Observe the not infrequent excentric position of the nucleus.

4. Place on the centre of a *very clean* cover-slip a fresh drop of blood from a frog just killed. Rapidly and yet carefully place on a glass slide (also scrupulously clean) the cover-slip, with the drop of blood undermost, and with the point of a needle gently move about until the drop is spread over its whole area. Cement with paraffin A. (which melts at 40° C) in the following manner. With a morsel of blotting paper dry if necessary the slide at the edges of the cover-slip. Keep the cover-slip in place by gently holding a needle against one edge, and with a small brush, brush

very carefully the melted paraffin over the edges all round. The paraffin need not extend more than $\frac{1}{8}$ or $\frac{1}{8}$ inch over the cover-slip. In a few minutes the paraffin becomes solid, and thus the fluid underneath is protected from evaporation.

5. Neglecting the red corpuscles, search, in the spaces where these are scanty, for the **colourless corpuscles.** Be careful not to confound with the colourless corpuscles any of the granular nuclei which may have escaped from the red corpuscles. Observe that

    a.   They are much fewer than the red.

    b.   They are smaller than the red, but not uniform in size.

    c.   They are of variable form, sometimes spherical but more frequently of irregular outline, and marked with protuberances.

    d.   They are colourless and granular. Some are much more coarsely granular than others.

    e.   They possess a transparent nucleus of round or irregular shape, sometimes more than one: since the refractive index of the nucleus differs very slightly from that of the surrounding protoplasm, its outline is seldom visible except when the corpuscle is very flat. Do not confound a heap of granules or a protuberance with the nucleus.

6. Examine several of the colourless corpuscles until you find one actively engaged in *amœboid movements.* Watch the movements carefully, sketching the outline of the corpuscle every twenty seconds. If the movements are not satisfactory, warm the slide on the hot stage.   (Hdb. p. 7.)

7. Take a fresh drop. Do not cement. Having found
    a characteristic white corpuscle, irrigate very cautiously
    with dilute acetic acid as in § 3, keeping the corpuscle
    in view. The corpuscle, when the acid reaches it,
    becomes more transparent, and a granular nucleus,
    often irregular or lobed, comes into view.

B. HUMAN BLOOD.

1. Take a drop of blood from the finger and cover as
    speedily as possible with a cover-slip. The glasses
    should be perfectly clean, and the layer of blood so
    thin that the red colour is hardly visible. It is as
    well to breathe on the under surface of the cover-slip
    immediately before putting it on. Observe the red
    corpuscles.

    a. They stick to one another, and, owing to their
        shape, usually in rouleaux.

    b. They roll about readily, as when the cover-slip is
        lightly touched.

    c. They are yellowish, circular bi-concave discs, so
        that when seen in profile they appear dumb-bell
        shaped.

    d. They appear homogeneous, and possess no nucleus.
        At a proper focus their centre is light; a little
        out of focus it appears dark.

    e. Towards the outside of the drop, where evapora-
        tion is going on, many of the red corpuscles are
        crenate.

    f. They are much smaller than the red corpuscles
        of the frog. Measure them.

2. Observe the white corpuscles. Look for them in the open spaces where the red corpuscles are few.

 a. They do not readily roll about.

 b. Their general characters are similar to those of the frog, which they also resemble in size.

3. Irrigate (see A. § 3) separate drops.

 a. With saline solution (5 p. c.). The red corpuscles become shrivelled and crenate.

 b. With water. The red corpuscles swell up, become spherical, and finally dissolve leaving behind a hardly visible ring of stroma.

 c. With dilute acetic acid. The red corpuscles undergo changes similar to those brought by water. Observe that there is no trace of a nucleus. The *white* corpuscles behave like those of the frog (A. § 7).

4. Examine a drop of the sediment which forms in blood repeatedly frozen and thawed. Observe the pale colourless rings and fragments of stroma.

DEMONSTRATIONS.

The amœboid movements of the white corpuscles and the formation and appearance of fibrin filaments under a high power.

LESSON III.

COAGULATION OF BLOOD.

1. Watch carefully the natural coagulation of freshly shed blood. This will be obtained by the demonstrator. Observe—*a.* The natural perfectly fluid stage. *b.* The viscid stage. *c.* The jelly stage. *d.* The appearance of the first drops of serum. *e.* The continued contraction of the fibrin, resulting in the ordinary clot and serum.

2. Whip a small quantity of freshly shed blood. Wash with water the clot so obtained. Note the quantity and observe the following general characters of fibrin.

   a. It appears under the microscope as a fine feltwork of granular fibrils.

   b. It is elastic.

   c. It is insoluble in water and in normal saline solution.

   d. It swells up and becomes transparent when treated with dilute hydrochloric acid; but it may be restored to its previous condition if the acid be neutralized.

   Leave in a test-tube a little of the whipped blood for twenty-four hours, and satisfy yourself that it undergoes no further coagulation.

3. Examine the plasma of horse's blood kept, by means
    of cold, from coagulating.   (Hdb. p. 167.)

    a. Transfer with a pipette 2 or 3 cc. of the plasma
    into a small test-tube.   Observe the coagulation
    and compare it with that of § 1. Avoid shaking.
    Probably the fibrin will adhere so strongly to the
    sides of the tube that little contraction will take
    place. On being freed from the glass it will
    contract.

    b. Dilute 1 cc. of the plasma with 50 cc. of distilled
    water or normal saline solution.   Carefully avoid
    shaking and leave it till the next day.   Observe
    the fine delicate fibrils of fibrin which are formed.

    c. Examine, without washing, the characters of the
    fibrin of *a* and *b*. They are best fitted to shew
    the microscopical characters; white corpuscles
    will be found entangled among the fibrils.

4. Examine the plasma of blood prevented from coagu-
    lating by the presence of neutral salts. (Hdb. p. 171.
    par. 10.)

    a. Remove 1 or 2 cc. carefully with a pipette,
    avoiding blood-corpuscles as much as possible,
    and dilute tenfold with water. The mixture will
    clot very speedily if placed in the warm chamber;
    less speedily if left at the ordinary temperature.

    b. Remove about 10 cc. into a small conical glass.
    Add gradually solid sodium chloride to excess,
    stirring no more than is necessary to assist the
    salt to dissolve. As the point of saturation is
    reached, a flaky precipitate makes its appearance.
    If the precipitate be plentiful, remove it with a
    spatula, put it on a small filter wetted with a *satu-*

rated solution of sodium chloride and wash with small quantities of the same: if the precipitate be small, decant it from the undissolved salt; filter and wash the precipitate on the filter paper with small quantities of a saturated sodium chloride solution. Dissolve the substance so obtained, the **plasmine** of Denis, in a small quantity of distilled water, and filter. Probably a portion of it will not dissolve, having already coagulated. The clear, colourless fluid filtrate will, if set on one side, clot. Avoid shaking after filtration. If a small quantity only of the fibrin factors be present, the fine threads of fibrin, as they are formed, are loosened by the shaking, and contract; thus the more easily recognized gelatinous stage is lost. *This operation is the more success-ful, the more rapidly it is carried on.*

5. To 1 cc. of hydrocele or other serous fluid which has been ascertained not to coagulate, or to have coagulated spontaneously, add 1 cc. of fresh blood-serum, gently mix, and put on one side.

After a while, possibly not until after twenty-four hours, the mixture will have coagulated. The coagulation will be more rapid in the warm chamber.

6. Dilute 10 or 15 cc. of fresh blood-serum with twenty times its volume of distilled water. Pass a brisk stream of carbonic acid through it for half an hour or longer. The mixture will become turbid and opaque. After standing for twelve hours a white amorphous precipitate will have settled to the bottom of the vessel. Siphon off all the clear supernatant fluid. (Fill up the vessel with water charged with carbonic acid, let it stand till the precipitate has again settled, and

again decant. This is for the purpose of still further getting rid of the serum, and may, if time presses, be omitted.)

The precipitate is for the most part **fibrinoplastin**. Add to the fluid containing the precipitate in suspension a drop or two of a 10 p. c. solution of chloride of sodium, and shake gently. The greater part of the precipitate will dissolve, fibrinoplastin being soluble in dilute solutions of sodium chloride. Filter, and let the clear filtrate stand. It will not coagulate spontaneously.

Fibrinoplastin may also be prepared from blood-serum by saturation with sodium chloride, as in § 4 *b*.

7. Add a small quantity of fibrinoplastin solution to hydrocele fluid. Coagulation will result.

8. Treat 10 cc. of hydrocele or pericardial fluid with sodium chloride to saturation, and proceed as in § 4 *b*; a precipitate, very similar in appearance to plasmine, will be obtained, but its solutions do not coagulate spontaneously. It is **fibrinogen**.

Fibrinogen may also be prepared by the carbonic acid method, as fibrinoplastin in § 6, but the salt method is preferable.

9. Add to 1 cc. of a strong solution of fibrinogen an equal volume of blood-serum, and set aside; coagulation will take place.

10. General characters of serum **albumin** (Hdb. 423 §§ 7, 8, 9). Dilute serum tenfold with water. Of this dilute serum

    a. Place 10 cc. in a test-tube with a thermometer and gradually heat in water-bath. Observe the

temperature (about 70° C.) at which the fluid be-
comes milky, and that at which coagulation is
complete.

b.   Take another 10 cc. and add a few drops of
strong nitric acid. The albumin is coagulated.

c.   To a third 10 cc. add 1 cc. of acetic acid and then
a strong solution of potassium ferrocyanide. The
albumin is precipitated.

11.   Tests for proteids (Hdb. p. 426, §§ 10, 11, 12). Dilute
serum twenty-fold with water.

a.   Xanthoproteic reaction.   Take a little of the
dilute serum, add a few drops of nitric acid, and
boil.   The white precipitate of albumin at first
formed becomes yellow, or if the quantity of
albumin present is small, dissolves, forming a
yellow solution.   Cool, and when cold add am-
monia; the yellow is turned to orange.

b.   To another small quantity of the serum add a few
drops of Millon's re-agent and boil for some
minutes. The fluid and the precipitate (if any
be formed) will become pink or rosy.

c.   Add a drop of cupric sulphate solution to an ex-
cess of liquor sodæ. To the blue solution so
formed add a drop of dilute serum, and warm;
the fluid will become violet.

DEMONSTRATIONS.

1.   Coagulation of blood over mercury without access of
air.

2.   Fluidity of blood in living blood vessels.

3.   Coagulation of blood within the vessels during life on
foreign bodies or in injured blood vessels.

LESSON IV.

CARTILAGE, BONE, AND TEETH.

A. HYALINE CARTILAGE.

1. Snip off a piece of the free edge of any of the thin
   cartilages projecting from the sternum or shoulder-
   girdle of a freshly killed young newt. Scrape off
   with a scalpel any tissue covering it. Mount it in
   normal saline solution[1] and note that

   a. The **matrix** is studded with **corpuscles** or **cells.**

   b. Each corpuscle consists of a round or oval mass
      of more or less coarsely granular **protoplasm,**
      in which lies a relatively large **nucleus.**

   c. Most of the cells entirely fill up the cavities in
      which they lie.

   d. At the extreme edge some cavities from which
      the cells have fallen out may generally be seen.

   e. Except at the edge, the cells are two or more
      layers deep.

   f. The matrix is hyaline or faintly granular.

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[1] In this and the following Lessons whenever a tissue is directed to be
mounted in normal saline solution it is to be understood that if either fresh
aqueous humour, or fresh blood-serum are obtainable, it is to be preferred.

2.   Irrigate the piece with acetic acid 5 p.c.; the nucleus
will become more granular and very distinct; some
few granules will be seen around it in the proto-
plasm, which otherwise has become clear and hardly
distinguishable.

Irrigate the same piece with spirit; the protoplasm
will again become distinct and granular, thus partly
hiding the nucleus, and will shrink, leaving a clear
space between itself and the matrix. The cartilage
will appear to the naked eye more opaque.

3.   Immerse it in hæmatoxylin for about five minutes,
place it on a slide, and wash with spirit to remove
any colouring matter simply adhering to the tissue.
If the cartilage has been stained too deeply, soak up
as much as possible of the spirit with blotting paper
and add a drop of acetic acid 5 p.c. until it is of a
light purple or violet colour. Soak up the acetic acid,
wash the tissue once more with spirit, and mount it
in glycerine. Observe that the matrix stains slightly,
the protoplasm of the cells more so, and the nuclei
most of all. The spaces between the shrunk cor-
puscles and the matrix are now very distinct, as are
also such empty cavities as happen to be present.

4.   Cut as thin a tranverse section as possible of the
articular end (preserved in Müller's fluid) of any bone
of a young animal. The cutting of the section will
be rendered easier if a small piece of the cartilage be
previously "imbedded" in the following manner. At
one end of a small oblong block of paraffin *B* scoop
out a small hole; and place the piece of cartilage in
the hole with that surface uppermost from which the
sections are to be taken. Pour over it slowly and
carefully a little of the paraffin mixture heated so as

to be just melted; keeping, if necessary, the tissue in its proper position by means of a heated needle; when the whole is thoroughly solid pare down the paraffin to the surface of the cartilage, at the same time bevelling the edges. In cutting the sections, cover the tissue and the blade of the razor with spirit (for convenience from a wash-bottle) and float the sections by means of a gentle stream of spirit into a watch-glass. With the platinum "lifter" transfer them to hæmatoxylin and treat as in § 3. Observe that

> The cells are smaller than in § 1, and much more scattered, since more matrix is formed between them. They are more irregular, often angular, in form, and those on the outside are seen to be flattened parallel to the surface. Otherwise the general features are the same.

5. Cut thin sections of costal cartilage fresh or preserved in Müller's fluid, imbedding if necessary, and observe that

   a. The protoplasm of the cells frequently contains large granules and oil-drops, from having undergone degeneration. Distinguish between a large oil-drop (very highly refractive) and a nucleus.

   b. The cells are arranged in groups; each cell in a group is called a daughter-cell, all the daughter-cells in a group having arisen from the division of a previously existing cell called a mother-cell. Make out the partitions dividing the mother-cell. This can be best done in stained specimens.

   c. The matrix in places may be fibrillated or laminated. Add acetic acid; it will have no effect on this fibrillation, which thus differs from that of connective tissue (Lesson V. A. 2, b).

3—2

d. In places the matrix may be opaque with calcareous deposit, removable by dilute hydrochloric acid.

6. Take a small piece of the ear of a freshly killed mouse, remove the skin and scrape away the tissue surrounding the ear-cartilage. Mount the cartilage in normal saline solution.

Observe that the cavities of the cells, generally polygonal in form, are separated by very narrow partitions of matrix. From many of the cavities the corpuscles have disappeared altogether, leaving simply a honeycombed matrix. This form of cartilage is frequently called " parenchymatous."

B. ELASTIC CARTILAGE.

Cut sections of epiglottis or arytenoid cartilage, or of the ear-cartilage fresh, or preserved in Müller's fluid.

The cells are like those of hyaline cartilage, but in the matrix are a number of branching anastomosing fibres, unaffected by acetic acid. Frequently there is round each cell a clear transparent border, forming the wall of the cavity in which the cell lies.   •

C. BONE.

1. Examine with a low objective transverse sections of compact bone prepared by grinding down thin slices and mounting them in Canada balsam. Observe

a. The **Haversian systems,** each consisting of a Haversian canal, surrounded by concentric **lamellæ** marked with **lacunæ.**

b. Their arrangement; make out which is the inner and which the outer surface of the bone.

    c. Their variable size, and form, partly due to the plane in which they are cut, whether obliquely or at right angles.

    d. Where the balsam has run in and rendered the tissue transparent, the canals, not being filled either with air or débris, are no longer black, but appear as holes.

    e. The irregular Haversian spaces, seen towards the cavity of the bone.

    f. The circumferential and intersystemic lamellæ.

2. Examine the same with a higher objective, and observe more carefully

    a. The lacunæ, and their arrangement in the Haversian system.

    b. Their form; irregularly oval, generally narrow but varying in breadth according to the plane in which they are cut, since they are flattened ovals.

    c. Their blackness, due to their being filled with air or débris. Where the balsam has run in, they appear as cavities.

    d. Their **canaliculi,** branching, anastomosing, and running across the lamellæ from lacuna to lacuna. They are seen as black lines when filled with air, but are almost invisible when filled with Canada balsam.

3. Examine similar longitudinal sections, first with a low and then with a high objective, and compare them with the above. Observe

    a. The Haversian canals, cut longitudinally or obliquely.

    b. Their divisions or junctions.

>   *c.*   Their openings on to the surface of the bone and into the medullary cavity.
>
>   *d.*   The lacunæ, generally cut lengthways and therefore narrow, and mostly disposed parallel to the Haversian canals. In many spots they may be seen scattered irregularly.

  4. Prepare transverse and longitudinal sections of bone which has been treated with chromic acid. Stain with hæmatoxylin and mount in glycerine. Observe

>   *a.*   The Haversian systems, with the Haversian canals as before.
>
>   *b.*   The remains of capillaries and blood-vessels in the Haversian canals and spaces.
>
>   *c.*   The cell, consisting of protoplasm and nucleus present in each lacuna.
>
>   *d.*   The canaliculi, appearing as fine, hardly visible streaks, or when seen in section as dots.

D. TEETH.

  1. Examine with a low objective longitudinal sections of teeth prepared in the same manner as bone in C. Observe the **dentine** surrounding the pulp cavity, the **cement** or **crusta petrosa** covering the dentine of the fangs, and the **enamel** covering the dentine of the crown. Note the general arrangement of the dentinal tubules.

  2. Examine with a higher objective the same section, and study in detail.

  a. The **Dentine.**

>   *a.*   In the matrix, apparently homogeneous, are numerous dentinal tubules which run in a wavy course from the pulp-cavity outwards. These,

dividing as they go, and giving off many anasto-
mosing lateral branches, finally end either in
loops or in small irregular cavities, the so-called
interglobular spaces, on the surface of the dentine.

b. In some places the dentinal tubules are cut
transversely. Here the central dark spot indi-
cates the space formerly occupied by the dentinal
fibre, the ring round this is the dentinal sheath.

β. The **Cement** or **Crusta Petrosa.**

a. This differs little from bone, but Haversian canals
are generally absent.

b. The canaliculi of the lacunæ, next to the inter-
globular spaces, open into these, thus bringing
the dentinal fibres into connection with the bone-
corpuscles.

c. Where the cement is massive, wavy 'contour'
lines may be seen, indicating the successive
deposits.

γ. The **Enamel.**

The striated enamel fibres or prisms are arranged
in sets, perpendicular to the surface of the dentine.
In mounted specimens the indications of the
separate fibres are generally not well marked,
but are visible at places. The line of junction of
the enamel and dentine is generally very con-
spicuous, in consequence of a change of level
in the specimen, the dentine having been ground
thinner than the harder enamel. The enamel
is frequently split or cracked.

3. Examine prepared transverse sections of Teeth, taken
at different heights, and compare with the above.

LESSON V.

CONNECTIVE TISSUE.

A. Connective-tissue Fibres.

1. **Elastic Fibres.**

    a. Tease out in water a morsel of ligamentum nuchæ. It is almost entirely made up of rather large branching, and anastomosing, fibres with bold outlines, curling at their ends.

    b. Irrigate with acetic acid; the fibres are unaffected.

2. **Proper Fibrillæ of Connective Tissue.**

    a. Tease out in water (Hdb. p. 32) a *small* piece of fresh tendon, e.g. from one of the digits of a frog. It is made up of bundles of wavy, delicate fibrillæ, whose outline is hardly visible.

    b. Irrigate with acetic acid 5 p.c. The fibrillæ disappear, and the whole tissue swells up into a transparent gelatinous mass, in which one can hardly distinguish anything besides a few elastic fibres similar to those of A. 1, but much finer.

    c. Snip off a piece of subcutaneous intermuscular connective tissue from the frog. Spread it out

carefully on a glass slide with water, and avoid
as much as possible pulling it about. It is
composed of similar wavy bundles of fibrillæ
arranged as a meshwork; these are similarly
affected by acetic acid. The elastic fibres are
more abundant, and very much curled along
their course.

B. CONNECTIVE-TISSUE CORPUSCLES.

1. Take a piece of the thin expansion of a tadpole's tail
    preserved in chromic acid. Gently break a piece up
    in glycerine. Where the hexagonal cells of the
    epidermis have broken away, the homogeneous matrix
    may be seen, imbedded in which are many blood-
    vessels and a number of stellate cells.

    The larger dark stellate cells are pigment-cells, the
    smaller stellate cells are **fixed connective-tissue
    corpuscles,** each of which consists of a nucleus
    and a branched protoplasmic body. The processes
    divide much, but irregularly, and communicate with
    the processes of other cells.

2. Break off the tip of the tail of a young rat just
    killed, and pull out from the stump a bundle of fine
    tendons. Spread these over a glass slide from edge
    to edge, putting a drop of normal saline solution in
    the middle only, so that they will dry at the edges
    and remain on the stretch. Cover with normal solu-
    tion and cover-slip. On examination the bundles of
    wavy fibrillæ will be seen, but no corpuscles.

    Slowly irrigate with acetic acid 1 p.c., watching
    the bundles the while under a high objective. Be-
    tween the bundles of fibres, and sometimes apparently

over them, will be seen rows of cells. Note in each
the nucleus, the rectangular outline of the cell, and
the granular protoplasm. Indications of lateral pro-
cesses may be seen by careful focussing. After the
acetic acid has been added for some little time the
protoplasm of the cells becomes very indistinct, and
between the swollen bundles scarcely anything is seen
but rows of irregularly shaped nuclei.

3.   Snip off a small piece of subcutaneous tissue in a new-
born mammal, and spread it carefully on a glass slide
without teasing; cover it with normal saline solution
and a cover-slip.

In the spaces where the fibrillæ are scanty will be
seen some pale, granular, branched cells, not unlike
those of B. 1.

Irrigate gently with acetic acid 1 p.c. the nuclei
of these connective-tissue corpuscles, each, surrounded
by an irregular-shaped mass of protoplasm, will come
distinctly into view.

4.   Take a frog, of which the brain and spinal cord have
been destroyed, squeeze the side of the head gently to
make the eye bulge out; then with a sharp scalpel
with one slice cut off the cornea. If any blood has
accidentally got on it, put it on a slide with a drop
of normal saline solution and gently brush it with a
camel's hair brush. Put it in gold chloride ·5 p.c.
solution till it is of a pale yellow colour, wash well,
transfer to water just acidulated with acetic acid,
and expose to light. When the cornea has become
of a weak blue-violet colour, put it on a slide with
glycerine, and brush or scrape both surfaces to remove
the epithelium, then mount in glycerine and look at
it first with a low, and then with a high objective.

Where the hexagonal surface epithelium has been removed, there will be seen stained connective-tissue corpuscles, with numerous fine processes branching and anastomosing with each other.

C. PIGMENT-CELLS.

Pin out on a frog-board one of the webs between the toes of the same frog, and observe first with a low and then with a high objective. There will be seen large corpuscles loaded with dark pigment, and possessing numerous branched processes. In some places the pigmented cells will appear as round dots, the processes in this case having been retracted. Every intermediate stage between these two states may be observed by watching from time to time.

D. FAT-CELLS.

1. Cut out a small piece of the omentum or subcutaneous tissue of a mammal, from a part containing comparatively little fat. Spread it out on a slide, and cover with normal saline solution and a cover-slip. Examine with a low objective. Observe the groups of highly refractive fat-cells.

2. Examine with a high objective. Observe the individual fat-cells, their variable size, the apparent absence of a nucleus, and the fibres of connective tissue passing between and over them.

3. Take a small piece of adipose tissue, which has been kept for some time in alcohol, and place it in hæmatoxylin until it has become well stained. Wash it with spirit, place it on a glass slide, and tease it out if it is too thick. Remove as much of the spirit

as possible with blotting paper. Cover the tissue with a mixture of carbolic acid (1 part) and turpentine (4 parts), and let it remain until it is completely transparent, changing the fluid and gently warming if necessary. Remove the excess of turpentine and cover the tissue with a drop of Canada balsam and put on a cover-slip.

Observe the groups of cells from which the fat has been thus removed. Note the shrunken outlines of the cells, and the presence in each cell of a nucleus and of a small amount of protoplasm, both of which are stained with hæmatoxylin.

4. Snip off a small portion of the gelatinous material from the orbit of the rabbit (Hdb. p. 44, Figs. 47, 48), and examine the transitional forms between connective-tissue corpuscles and fat-cells.

E. CONNECTIVE TISSUE with CARTILAGE-CORPUSCLES: FIBRO-CARTILAGE.

Imbed in paraffin *B* a small piece of mammalian intervertebral cartilage which has been hardened in chromic acid. Cut sections and stain them with hæmatoxylin. Observe

a. The fibrous connective-tissue, sometimes collected into bands, sometimes forming a meshwork.

b. The ordinary connective-tissue corpuscles which occur in parts.

c. The cartilage-corpuscles; large cells with a distinct nucleus; they are often seen in groups.

LESSON VI.

STRUCTURE OF CONTRACTILE TISSUES.

1. Scrape the mucous membrane of the throat or roof of a frog's mouth, which has been treated with osmic acid 1 p. c. for twenty-four hours. Tease out the flakes so obtained in a drop of water, and examine with avoidance of pressure. Groups of **ciliated cells**, intermingled with goblet-cells will be observed. Neglect the latter; note in the former

    a. The shape of the cell; it is more or less conical, often with a branching or irregular apex.

    b. The cilia on the base. Seen in profile, they appear to form a row, but when a cell is obtained in such a position that it is viewed from above, the cilia, foreshortened into mere dots, are seen to be spread over the whole surface of the base.

    c. The nucleus, the granular protoplasm, and the hyaline border just below the cilia.

2. Scrape the same membrane in a recently killed frog, and gently tease the scrapings in normal saline solution. Note

    a. The movements of the cilia, watching especially those in which the action is becoming faint.

    b. The results of ciliary action: granules and blood-corpuscles are driven along. Detached cells may also be seen carried about by the action of their own cilia.

    c. The contracted, almost globular form of the cells which have been set free.

3. Tear off the smallest possible strip of a **frog's muscle,** preserved in alcohol, add a drop of acetic acid, and tease it out as finely as possible in water. Note

    a. The varying size of the elementary fibres.

    b. The striation of the fibres: alternate dim and bright cross-bands passing through the whole thickness of the fibre.

    c. The breaking up of the fibre into fibrillæ, the latter also striated. Try to obtain as fine fibrillæ as possible. The fibres sometimes split transversely into discs; the surfaces of the discs appear dotted.

4. Remove carefully, with sharp scissors, one of the thin muscles from the neck of a frog, and mount it in normal saline solution, avoiding pressure. The fibres will for a brief period be seen in a normal condition. In many, the alternate dim and bright striæ will be very distinct; in others the transverse markings will be more or less obscured by an appearance of longitudinal fibrillation. Occasionally fibres are met with, having only a confused granular aspect. If the preparation has been successfully made, waves of contraction may, at times, be seen to travel along the fibres. At the contracted part, the fibre is, for the time being, thicker, and the striæ set closer together.

5. Irrigate with distilled water; the fibres will become

more opaque, and after a while, a pale homogeneous membrane, the sarcolemma, will bulge out at one spot or another.

6. Irrigate with dilute acetic acid. Observe the elongated nuclei disposed lengthways, with lines of granules proceeding from their ends.

7. Make a thin transverse section of muscle hardened in chromic acid.
   Observe the cut ends of the fibres, and their arrangement in bundles.

8. Repeat § 3 on **mammalian muscle.** The fibres are much more uniform in size, but the same general features may be observed.

9. Tease out in water a strip of **plain muscular tissue,** taken from a stomach or intestine preserved in potassium bichromate. Note

   a. The isolated fibre-cells. These are long, and, when not broken off in the preparation, fusiform masses of hyaline or faintly granular protoplasm. They appear very much thinner when seen in one direction than in another; that is to say, they are much flattened. In the middle of each fibre an oval nucleus may with difficulty be seen. In the neighbourhood of the nucleus, especially at its ends, granules are more abundant in the protoplasm.

   b. The arrangement of the fibre-cells in bands and sheets: the fibres may frequently be seen projecting like a palisade from the torn end of a band.

10. Examine a strip of plain muscle from a perfectly fresh intestine, having teased it out *very gently* in

normal saline solution. The individual fibre-cells are seen with great difficulty, and when visible are often contracted.

11. Add acetic acid. Note in each the nucleus which now appears rod-shaped, and contains one or sometimes two dark spots, the nucleoli.

12. Tease out thoroughly a small portion of **cardiac muscle** preserved in potassium bichromate. Note that

    a. The striation is generally obscure.

    b. No sarcolemma is present.

    c. The fibres may frequently be seen branching and anastomosing.

    d. Nuclei are present disposed with considerable regularity. Around each nucleus is an area of contractile substance (muscle-cell) marked out by obscure partitions from neighbouring areas.

The nuclei will become more evident if the tissue be stained with hæmatoxylin.

DEMONSTRATIONS.

The structure of a living muscular fibre of Hydrophilus or Dytiscus as seen under a very high power.

The appearances of muscular fibre under polarised light.

LESSON VII.

PROPERTIES OF CONTRACTILE TISSUE.

1. **Constant Current.**

Place on its belly a frog, the brain and spinal cord of which have been destroyed, and make a longitudinal incision through the skin of the back of the thigh. Separate the large semi-membranosus muscle which lies on the inside of thigh, from the smaller biceps muscle which lies more to the outside. (Compare *Elementary Biology*, XIII. E. 4, p. 221.) The sciatic nerve with the femoral vessels will come into view.

Taking as much care as possible not to injure the nerve, isolate it by tearing away carefully the surrounding connective tissue.

Place under the nerve the shielded electrodes (Hdb. fig. 271), so that the nerve, and the nerve only, touches the platinum wires.

Connect the wires of the electrodes with a Du Bois Reymond's Key (Hdb. fig. 300), and this again with a battery of two Daniell's[1] cells. The key short-circuits the battery. Hence when the key is opened, the current is thrown into the nerve.

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[1] In the following experiments, for convenience in description, Daniell's cells and Du Bois Reymond's key are recommended to be used. Any other battery of corresponding strength, or any other form of key, will serve.

temperature (about 70° C.) at which the fluid be-
comes milky, and that at which coagulation is
complete.

b.   Take another 10 cc. and add a few drops of
strong nitric acid.   The albumin is coagulated.

c.   To a third 10 cc. add 1 cc. of acetic acid and then
a strong solution of potassium ferrocyanide.   The
albumin is precipitated.

11.   Tests for proteids (Hdb. p. 426, §§ 10, 11, 12).   Dilute
serum twenty-fold with water.

a.   Xanthoproteic reaction.   Take a little of the
dilute serum, add a few drops of nitric acid, and
boil.   The white precipitate of albumin at first
formed becomes yellow, or if the quantity of
albumin present is small, dissolves, forming a
yellow solution.   Cool, and when cold add am-
monia; the yellow is turned to orange.

b.   To another small quantity of the serum add a few
drops of Millon's re-agent and boil for some
minutes.   The fluid and the precipitate (if any
be formed) will become pink or rosy.

c.   Add a drop of cupric sulphate solution to an ex-
cess of liquor sodæ.   To the blue solution so
formed add a drop of dilute serum, and warm;
the fluid will become violet.

DEMONSTRATIONS.

1.   Coagulation of blood over mercury without access of
air.

2.   Fluidity of blood in living blood vessels.

3.   Coagulation of blood within the vessels during life on
foreign bodies or in injured blood vessels.

LESSON IV.

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    b. Each corpuscle consists of a round or oval mass
    of more or less coarsely granular **protoplasm**,
    in which lies a relatively large **nucleus.**

    c. Most of the cells entirely fill up the cavities in
    which they lie.

    d. At the extreme edge some cavities from which
    the cells have fallen out may generally be seen.

    e. Except at the edge, the cells are two or more
    layers deep.

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mounted in normal saline solution it is to be understood that if either fresh
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2.   Irrigate the piece with acetic acid 5 p.c.; the nucleus
will become more granular and very distinct; some
few granules will be seen around it in the proto-
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Irrigate the same piece with spirit; the protoplasm
will again become distinct and granular, thus partly
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space between itself and the matrix. The cartilage
will appear to the naked eye more opaque.

3.   Immerse it in hæmatoxylin for about five minutes,
place it on a slide, and wash with spirit to remove
any colouring matter simply adhering to the tissue.
If the cartilage has been stained too deeply, soak up
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4.   Cut as thin a tranverse section as possible of the
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carefully a little of the paraffin mixture heated so as

to be just melted; keeping, if necessary, the tissue in its proper position by means of a heated needle; when the whole is thoroughly solid pare down the paraffin to the surface of the cartilage, at the same time bevelling the edges. In cutting the sections, cover the tissue and the blade of the razor with spirit (for convenience from a wash-bottle) and float the sections by means of a gentle stream of spirit into a watch-glass. With the platinum "lifter" transfer them to hæmatoxylin and treat as in § 3. Observe that

The cells are smaller than in § 1, and much more scattered, since more matrix is formed between them. They are more irregular, often angular, in form, and those on the outside are seen to be flattened parallel to the surface. Otherwise the general features are the same.

5. Cut thin sections of costal cartilage fresh or preserved in Müller's fluid, imbedding if necessary, and observe that

   a. The protoplasm of the cells frequently contains large granules and oil-drops, from having undergone degeneration. Distinguish between a large oil-drop (very highly refractive) and a nucleus.

   b. The cells are arranged in groups; each cell in a group is called a daughter-cell, all the daughter-cells in a group having arisen from the division of a previously existing cell called a mother-cell. Make out the partitions dividing the mother-cell. This can be best done in stained specimens.

   c. The matrix in places may be fibrillated or laminated. Add acetic acid; it will have no effect on this fibrillation, which thus differs from that of connective tissue (Lesson V. A. 2, *b*).

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>   *d.*   In places the matrix may be opaque with cal-
>   careous deposit, removable by dilute hydrochloric
>   acid.

6. Take a small piece of the ear of a freshly killed mouse,
    remove the skin and scrape away the tissue surround-
    ing the ear-cartilage.   Mount the cartilage in normal
    saline solution.

    Observe that the cavities of the cells, generally
    polygonal in form, are separated by very narrow par-
    titions of matrix.   From many of the cavities the
    corpuscles have disappeared altogether, leaving simply
    a honeycombed matrix.   This form of cartilage is
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    b.   Their arrangement; make out which is the inner
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c.   Their variable size, and form, partly due to the plane in which they are cut, whether obliquely or at right angles.

d.   Where the balsam has run in and rendered the tissue transparent, the canals, not being filled either with air or débris, are no longer black, but appear as holes.

e.   The irregular Haversian spaces, seen towards the cavity of the bone.

f.   The circumferential and intersystemic lamellæ.

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a.   The lacunæ, and their arrangement in the Haversian system.

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d.   Their **canaliculi,** branching, anastomosing, and running across the lamellæ from lacuna to lacuna. They are seen as black lines when filled with air, but are almost invisible when filled with Canada balsam.

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  c. Their openings on to the surface of the bone and into the medullary cavity.

  d. The lacunæ, generally cut lengthways and therefore narrow, and mostly disposed parallel to the Haversian canals. In many spots they may be seen scattered irregularly.

 4. Prepare transverse and longitudinal sections of bone which has been treated with chromic acid. Stain with hæmatoxylin and mount in glycerine. Observe

  a. The Haversian systems, with the Haversian canals as before.

  b. The remains of capillaries and blood-vessels in the Haversian canals and spaces.

  c. The cell, consisting of protoplasm and nucleus present in each lacuna.

  d. The canaliculi, appearing as fine, hardly visible streaks, or when seen in section as dots.

D, TEETH.

 1. Examine with a low objective longitudinal sections of teeth prepared in the same manner as bone in C. Observe the **dentine** surrounding the pulp cavity, the **cement** or **crusta petrosa** covering the dentine of the fangs, and the **enamel** covering the dentine of the crown. Note the general arrangement of the dentinal tubules.

 2. Examine with a higher objective the same section, and study in detail.

 a. The **Dentine.**

  a. In the matrix, apparently homogeneous, are numerous dentinal tubules which run in a wavy course from the pulp-cavity outwards. These,

dividing as they go, and giving off many anasto-
mosing lateral branches, finally end either in
loops or in small irregular cavities, the so-called
interglobular spaces, on the surface of the dentine.

b. In some places the dentinal tubules are cut
transversely. Here the central dark spot indi-
cates the space formerly occupied by the dentinal
fibre, the ring round this is the dentinal sheath.

β. The **Cement** or **Crusta Petrosa.**

a. This differs little from bone, but Haversian canals
are generally absent.

b. The canaliculi of the lacunæ, next to the inter-
globular spaces, open into these, thus bringing
the dentinal fibres into connection with the bone-
corpuscles.

c. Where the cement is massive, wavy 'contour'
lines may be seen, indicating the successive
deposits.

γ. The **Enamel.**

The striated enamel fibres or prisms are arranged
in sets, perpendicular to the surface of the dentine.
In mounted specimens the indications of the
separate fibres are generally not well marked,
but are visible at places. The line of junction of
the enamel and dentine is generally very con-
spicuous, in consequence of a change of level
in the specimen, the dentine having been ground
thinner than the harder enamel. The enamel
is frequently split or cracked.

3. Examine prepared transverse sections of Teeth, taken
at different heights, and compare with the above.

LESSON V.

CONNECTIVE TISSUE.

A. CONNECTIVE-TISSUE FIBRES.

1. **Elastic Fibres.**

   a. Tease out in water a morsel of ligamentum nuchæ. It is almost entirely made up of rather large branching, and anastomosing, fibres with bold outlines, curling at their ends.

   b. Irrigate with acetic acid; the fibres are unaffected.

2. **Proper Fibrillæ of Connective Tissue.**

   a. Tease out in water (Hdb. p. 32) a *small* piece of fresh tendon, e.g. from one of the digits of a frog. It is made up of bundles of wavy, delicate fibrillæ, whose outline is hardly visible.

   b. Irrigate with acetic acid 5 p.c. The fibrillæ disappear, and the whole tissue swells up into a transparent gelatinous mass, in which one can hardly distinguish anything besides a few elastic fibres similar to those of A. 1, but much finer.

   c. Snip off a piece of subcutaneous intermuscular connective tissue from the frog. Spread it out

carefully on a glass slide with water, and avoid as much as possible pulling it about. It is composed of similar wavy bundles of fibrillæ arranged as a meshwork; these are similarly affected by acetic acid. The elastic fibres are more abundant, and very much curled along their course.

B. Connective-tissue Corpuscles.

1. Take a piece of the thin expansion of a tadpole's tail preserved in chromic acid. Gently break a piece up in glycerine. Where the hexagonal cells of the epidermis have broken away, the homogeneous matrix may be seen, imbedded in which are many blood-vessels and a number of stellate cells.

    The larger dark stellate cells are pigment-cells, the smaller stellate cells are **fixed connective-tissue corpuscles,** each of which consists of a nucleus and a branched protoplasmic body. The processes divide much, but irregularly, and communicate with the processes of other cells.

2. Break off the tip of the tail of a young rat just killed, and pull out from the stump a bundle of fine tendons. Spread these over a glass slide from edge to edge, putting a drop of normal saline solution in the middle only, so that they will dry at the edges and remain on the stretch. Cover with normal solution and cover-slip. On examination the bundles of wavy fibrillæ will be seen, but no corpuscles.

    Slowly irrigate with acetic acid 1 p. c., watching the bundles the while under a high objective. Between the bundles of fibres, and sometimes apparently

over them, will be seen rows of cells. Note in each
the nucleus, the rectangular outline of the cell, and
the granular protoplasm. Indications of lateral pro-
cesses may be seen by careful focussing. After the
acetic acid has been added for some little time the
protoplasm of the cells becomes very indistinct, and
between the swollen bundles scarcely anything is seen
but rows of irregularly shaped nuclei.

3.   Snip off a small piece of subcutaneous tissue in a new-
born mammal, and spread it carefully on a glass slide
without teasing; cover it with normal saline solution
and a cover-slip.

In the spaces where the fibrillæ are scanty will be
seen some pale, granular, branched cells, not unlike
those of B. 1.

Irrigate gently with acetic acid 1 p.c. the nuclei
of these connective-tissue corpuscles, each, surrounded
by an irregular-shaped mass of protoplasm, will come
distinctly into view.

4.   Take a frog, of which the brain and spinal cord have
been destroyed, squeeze the side of the head gently to
make the eye bulge out; then with a sharp scalpel
with one slice cut off the cornea. If any blood has
accidentally got on it, put it on a slide with a drop
of normal saline solution and gently brush it with a
camel's hair brush. Put it in gold chloride ·5 p.c.
solution till it is of a pale yellow colour, wash well,
transfer to water just acidulated with acetic acid,
and expose to light. When the cornea has become
of a weak blue-violet colour, put it on a slide with
glycerine, and brush or scrape both surfaces to remove
the epithelium, then mount in glycerine and look at
it first with a low, and then with a high objective.

Where the hexagonal surface epithelium has been removed, there will be seen stained connective-tissue corpuscles, with numerous fine processes branching and anastomosing with each other.

C. PIGMENT-CELLS.

Pin out on a frog-board one of the webs between the toes of the same frog, and observe first with a low and then with a high objective. There will be seen large corpuscles loaded with dark pigment, and possessing numerous branched processes. In some places the pigmented cells will appear as round dots, the processes in this case having been retracted. Every intermediate stage between these two states may be observed by watching from time to time.

D. FAT-CELLS.

1. Cut out a small piece of the omentum or subcutaneous tissue of a mammal, from a part containing comparatively little fat. Spread it out on a slide, and cover with normal saline solution and a cover-slip. Examine with a low objective. Observe the groups of highly refractive fat-cells.

2. Examine with a high objective. Observe the individual fat-cells, their variable size, the apparent absence of a nucleus, and the fibres of connective tissue passing between and over them.

3. Take a small piece of adipose tissue, which has been kept for some time in alcohol, and place it in hæmatoxylin until it has become well stained. Wash it with spirit, place it on a glass slide, and tease it out if it is too thick. Remove as much of the spirit

as possible with blotting paper. Cover the tissue with a mixture of carbolic acid (1 part) and turpentine (4 parts), and let it remain until it is completely transparent, changing the fluid and gently warming if necessary. Remove the excess of turpentine and cover the tissue with a drop of Canada balsam and put on a cover-slip.

Observe the groups of cells from which the fat has been thus removed. Note the shrunken outlines of the cells, and the presence in each cell of a nucleus and of a small amount of protoplasm, both of which are stained with hæmatoxylin.

4. Snip off a small portion of the gelatinous material from the orbit of the rabbit (Hdb. p. 44, Figs. 47, 48), and examine the transitional forms between connective-tissue corpuscles and fat-cells.

E. CONNECTIVE TISSUE with CARTILAGE-CORPUSCLES: FIBRO-CARTILAGE.

Imbed in paraffin *B* a small piece of mammalian intervertebral cartilage which has been hardened in chromic acid. Cut sections and stain them with hæmatoxylin. Observe

a. The fibrous connective-tissue, sometimes collected into bands, sometimes forming a meshwork.

b. The ordinary connective-tissue corpuscles which occur in parts.

c. The cartilage-corpuscles; large cells with a distinct nucleus; they are often seen in groups.

LESSON VI.

STRUCTURE OF CONTRACTILE TISSUES.

1. Scrape the mucous membrane of the throat or roof of a frog's mouth, which has been treated with osmic acid 1 p. c. for twenty-four hours. Tease out the flakes so obtained in a drop of water, and examine with avoidance of pressure. Groups of **ciliated cells**, intermingled with goblet-cells will be observed. Neglect the latter; note in the former

   a, The shape of the cell; it is more or less conical, often with a branching or irregular apex.

   b. The cilia on the base. Seen in profile, they appear to form a row, but when a cell is obtained in such a position that it is viewed from above, the cilia, foreshortened into mere dots, are seen to be spread over the whole surface of the base.

   c. The nucleus, the granular protoplasm, and the hyaline border just below the cilia,

2. Scrape the same membrane in a recently killed frog, and gently tease the scrapings in normal saline solution. Note

   a, The movements of the cilia, watching especially those in which the action is becoming faint.

b. The results of ciliary action: granules and blood-corpuscles are driven along. Detached cells may also be seen carried about by the action of their own cilia.

c. The contracted, almost globular form of the cells which have been set free.

3. Tear off the smallest possible strip of a **frog's muscle,** preserved in alcohol, add a drop of acetic acid, and tease it out as finely as possible in water. Note

a. The varying size of the elementary fibres.

b. The striation of the fibres: alternate dim and bright cross-bands passing through the whole thickness of the fibre.

c. The breaking up of the fibre into fibrillæ, the latter also striated. Try to obtain as fine fibrillæ as possible. The fibres sometimes split transversely into discs; the surfaces of the discs appear dotted.

4. Remove carefully, with sharp scissors, one of the thin muscles from the neck of a frog, and mount it in normal saline solution, avoiding pressure. The fibres will for a brief period be seen in a normal condition. In many, the alternate dim and bright striæ will be very distinct; in others the transverse markings will be more or less obscured by an appearance of longitudinal fibrillation. Occasionally fibres are met with, having only a confused granular aspect. If the preparation has been successfully made, waves of contraction may, at times, be seen to travel along the fibres. At the contracted part, the fibre is, for the time being, thicker, and the striæ set closer together.

5. Irrigate with distilled water; the fibres will become

more opaque, and after a while, a pale homogeneous membrane, the sarcolemma, will bulge out at one spot or another.

6. Irrigate with dilute acetic acid. Observe the elongated nuclei disposed lengthways, with lines of granules proceeding from their ends.

7. Make a thin transverse section of muscle hardened in chromic acid.
    Observe the cut ends of the fibres, and their arrangement in bundles.

8. Repeat § 3 on **mammalian muscle.** The fibres are much more uniform in size, but the same general features may be observed.

9. Tease out in water a strip of **plain muscular tissue,** taken from a stomach or intestine preserved in potassium bichromate. Note

    a. The isolated fibre-cells. These are long, and, when not broken off in the preparation, fusiform masses of hyaline or faintly granular protoplasm. They appear very much thinner when seen in one direction than in another; that is to say, they are much flattened. In the middle of each fibre an oval nucleus may with difficulty be seen. In the neighbourhood of the nucleus, especially at its ends, granules are more abundant in the protoplasm.

    b. The arrangement of the fibre-cells in bands and sheets: the fibres may frequently be seen projecting like a palisade from the torn end of a band.

10. Examine a strip of plain muscle from a perfectly fresh intestine, having teased it out *very gently* in

normal saline solution. The individual fibre-cells are seen with great difficulty, and when visible are often contracted.

11. Add acetic acid. Note in each the nucleus which now appears rod-shaped, and contains one or sometimes two dark spots, the nucleoli.

12. Tease out thoroughly a small portion of **cardiac muscle** preserved in potassium bichromate. Note that

   a. The striation is generally obscure.

   b. No sarcolemma is present.

   c. The fibres may frequently be seen branching and anastomosing.

   d. Nuclei are present disposed with considerable regularity. Around each nucleus is an area of contractile substance (muscle-cell) marked out by obscure partitions from neighbouring areas.

   The nuclei will become more evident if the tissue be stained with hæmatoxylin.

DEMONSTRATIONS.

The structure of a living muscular fibre of Hydrophilus or Dytiscus as seen under a very high power.

The appearances of muscular fibre under polarised light.

LESSON VII.

PROPERTIES OF CONTRACTILE TISSUE.

1. Constant Current.

Place on its belly a frog, the brain and spinal cord of which have been destroyed, and make a longitudinal incision through the skin of the back of the thigh. Separate the large semi-membranosus muscle which lies on the inside of thigh, from the smaller biceps muscle which lies more to the outside. (Compare *Elementary Biology*, XIII. E. 4, p. 221.) The sciatic nerve with the femoral vessels will come into view.

Taking as much care as possible not to injure the nerve, isolate it by tearing away carefully the surrounding connective tissue.

Place under the nerve the shielded electrodes (Hdb. fig. 271), so that the nerve, and the nerve only, touches the platinum wires.

Connect the wires of the electrodes with a Du Bois Reymond's Key (Hdb. fig. 300), and this again with a battery of two Daniell's[1] cells. The key short-circuits the battery. Hence when the key is opened, the current is thrown into the nerve.

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[1] In the following experiments, for convenience in description, Daniell's cells and Du Bois Reymond's key are recommended to be used. Any other battery of corresponding strength, or any other form of key, will serve.

L.   4

The leg being perfectly at rest, and the key closed, open the key, and after a few seconds close it again. A movement in the leg caused by contractions in the muscles to which the nerve is distributed, will be seen when the key is either opened or shut, or on both occasions. Save in exceptional cases, *there is no movement during the passage of the current through the nerve, but only when the current is thrown into or shut off from it* (the current being supposed to be tolerably constant.) Do not repeat this experiment more than once or twice on the same nerve, lest it be too much exhausted for the succeeding observations.

2. Single Induction Shocks.

Now connect the wires of the electrodes with the binding screws of the secondary spiral of a Du Bois Reymond's induction machine (Hdb. p. 351), and one of the top screws of the same machine with one pole of a Daniell's cell, connecting the other top screw with a Du Bois Reymond's key, from which connect a wire to the remaining pole of the cell, so that when the key is put down, the current passes into the primary coil, and induces a momentary current in the secondary coil, and in the electrodes therewith connected. When the key is opened, the circuit is broken, the current disappears from the primary coil, and in so doing induces a momentary current in the secondary coil and the electrodes.

Place the marker at the fore part of the secondary coil at the mark 10 on the scale, and, the frog's leg being at rest, shut and open the key. At each shutting and opening of the key, i.e. at each momentary induced current, a single sharp movement of

the leg will take place. The 'single induction shock' causes a *single contraction* or *spasm* of the muscles of the leg.

Be careful to replace the nerve on the electrodes (without pinching it) if it slips off during the movements.

If good contractions are not obtained with the secondary coil at 10, push it up gradually over the primary coil, one division at a time.

3. Shut and open the key as rapidly as you can, for a few seconds. The contraction of the muscle taking longer time to develope than the induced current, each contraction will catch up its predecessor, and the leg will remain rigidly contracted, or nearly so, as long as the breaking and making of the primary current is kept up. In other words, *tetanus* is produced. This is however more easily brought about as follows.

4. **Interrupted Current.**

Remove the wires from the screws at the top of the primary coil to those at the base, thus putting into action the magnetic interruptor attached to the machine (Hdb. p. 351), using, however, the key as before. Remove the electrodes from the nerve while arranging the interruptor. The machine should work in such a way that immediately the key is put down the hammer at once is thrown into oscillations (as indicated by its noise). The current is made and broken at each oscillation. Having replaced the nerve on the electrodes, put down the key. Immediately that the sound of the hammer is heard, the leg is thrust out straight by the tetanic contractions of the muscle, and remains so as long as

the current continues to be thus made and broken.
Open the key, at once the limb becomes flaccid and
quiet.   The "interrupted current" should not be
applied for more than five seconds.

5.   **Chemical Stimulation.**

Dissect out the sciatic nerve up to the spinal column,
so as to have as long a piece as possible.   Let the
extreme end of the nerve dip into a saturated solu-
tion of sodium chloride; watch the leg.

In a variable time twitchings of the toes will be seen,
and after a while these will increase in vigour and
extent over the limb until the latter becomes almost
as rigid as in § 4.

6.   **Mechanical Stimulation.**

Cut off the part of the nerve which has been dipping
in the salt solution; the chemical stimulus being
thus removed, the leg will become flaccid again.

Pinch the remaining nerve sharply with a pair of
forceps several times.   At each pinch the muscles of
the leg will contract.   The pinching kills or injures
the part of the nerve pinched; hence it is necessary
to begin at the cut end, and work down towards the
muscle.

The gastrocnemius muscle of this leg may be used
for § 11, *b.*

7.   **Muscle Curve.**

From the other leg of the same frog get ready a
muscle-nerve preparation.   (Hdb. p. 343.)

Place the preparation in the moist chamber (Hdb.
pp. 344 and 348) with the nerve on a pair of electrodes.
Attach the tendon to the lever, load with 15 or 20
grammes, and bring the lever to mark on the revolv-
ing cylinder.

Connect the electrodes with the induction machine arranged for single induction shocks as in § 2.

Take tracings of single contractions or simple spasms of the muscle caused by simple induction shocks (coil at 10 to begin with for instance), using the swiftest speed of the cylinder. Observe the rapid but gradual rise to a maximum, passing almost immediately into a similar but less rapid fall, the later stages of the fall being distinctly more gradual than the early ones.

Note that the contractions caused by the opening or 'breaking' shock are, as a rule, greater than those caused by the closing or 'making' shock.

8. **Tetanus.**

Now arrange the induction machine for an interrupted current, (§ 4,) the secondary coil being at 15 or 20 to begin with.

Take a tracing of a tetanic contraction of a muscle, stimulating for not more than three or four seconds.

Observe the rise to a maximum at first rapid but afterwards more and more slow, the maintenance of the maximum during the application of the current, and the fall, at first rapid but afterwards more slow, after the removal of the current.

Watch during the tetanus the muscle itself as well as the curve.

9. Load the muscle with 50 grammes, allowing the lever to write on the stationary cylinder. Note the elasticity of the muscle. The weight when applied stretches the muscle to a certain extent, but when raised so as not to drag on the muscle, the latter returns, at once to almost, and eventually to quite, its former length. Stimulate with an interrupted current (coil say at

20).   Note the amount of contraction.   Repeat this with 100, and then with 200 grammes, using in each case the same strength of current for the same time, moving the cylinder with the hand so as to expose a fresh black surface for each observation.

Observe that with medium weights the work done (the product of the weight into the height) is greater than with very light or very heavy weights.

Observe also that the muscle gradually becomes exhausted, i. e. the contractions caused by the same stimulus gradually become less and less.

10.   Remove the magnetic interruptor from the primary circuit, and insert the oscillating rod. (Hdb. p. 353.)   Take tracings of the contractions so caused. Observe the gradual fusing of a series of single contraction-curves into the curve of tetanus.

11.   **Reaction of Muscle.**

a.   Remove the muscle from the moist chamber, place it on a *clean* glass slide, and apply the electrodes, connected with the induction machine arranged for an interrupted current, *directly* to the muscle.

Observe that the current applied directly to the muscle produces the same effect as when applied indirectly through the nerve.

Tetanize the muscle with currents of increasing strength until it is completely exhausted, pushing the secondary coil right up over the primary.

Cut the muscle across in half, with a *quite clean* scalpel, and press on one section a piece of faintly blue, on the other a piece of neutral

litmus paper. Both will be turned red where they come in contact with the muscle.

b. Dissect out the gastrocnemius from the leg used in §§ 1—6, and place it for five minutes in normal saline solution heated to 50° C.

Observe that it has become contracted and opaque, i.e. *rigor mortis* has set in.

Cut the muscle across, and test with litmus paper as in *a*. The muscle will be distinctly acid, even more so than the tetanized muscle in *a*.

c. Take a fresh living gastrocnemius from a frog just killed, cut it across as in *a* and *b*, and test the sections with neutral litmus paper. The muscle will be found neutral or faintly alkaline.

12. **Myosin.**

Take some muscle which has been minced and washed until all the substances soluble in water have been removed (Hdb. p. 452).

Rub up a small portion with an equal bulk of a 10 p. c. solution of sodium chloride and let it stand for twenty four hours. Press the glairy mass through a piece of linen, and then filter through a coarse filter. Let the filtrate drop into a tall jar containing some 500 cc. of distilled water. As each drop falls into the water it gives rise to a white flocculent precipitate. This is **myosin**, which, after standing for some hours, will settle at the bottom of the vessel.

Pour off the clear fluid, filtering if necessary, and determine that the myosin is soluble

a. In a 10 p. c. sodium chloride solution, the solution coagulating on being boiled,

  b.   In dilute acids and dilute alkalis, the solutions
        not coagulating on being boiled.

13. Syntonin.

Treat another portion of the same washed muscle
with ten times its bulk of hydrochloric acid 1 p. c.,
and place in the warm chamber, frequently shaking.
After three or four hours much of the muscle will
have disappeared.

Filter off the clear fluid, and carefully neutralize the
filtrate with a weak solution of sodium carbonate; a
copious bulky precipitate of **syntonin** takes place.
Separate the precipitate by filtration, avoiding ex-
posure to air as much as possible, and determine that

  a.   It is soluble in dilute acids and alkalis.
  b.   The acid and alkaline solutions are not coagu-
        lated by heat ;
  c.   The syntonin is precipitated from these solutions
        on careful neutralisation.
  d.   The precipitated syntonin *suspended* in water
        and boiled becomes coagulated, and is no longer
        soluble in dilute acids and alkalis.

DEMONSTRATIONS.

The natural electric currents of muscle.

The negative variation of the current attending a contrac-
tion of the muscle.

The rheoscopic frog.

Measurement of the latent period of contraction.

LESSON VIII.

STRUCTURE OF NERVOUS TISSUES.

A. SPINAL NERVES.

1. Cut off about one third of an inch of a perfectly fresh
   nerve (e.g. the sciatic of a frog), and place it on a glass
   slide *without any fluid*. With needles gently tease
   out one end into the shape of a fan; add a drop of
   normal saline solution, and cover with a cover-slip.
   Observe

   a. The **nerve-fibres** of variable size.

   b. In each the double contour, due to the **medullary
      sheath** or white substance of Schwann.

   c. The **primitive sheath;** this is seen with difficulty
      except at points where the medullary sheath is
      wanting.

   d. The connective tissue (perineurium) running
      amongst and around the nerve-fibres.

   e. Drops and fragments of the medullary sheath,
      extending from the cut ends of the fibres and
      shewing a double contour.

2. Cut off a similar piece of nerve and treat it in the same
   way, but instead of adding a normal solution, pour
   over the nerve a drop of chloroform, and immediately

cover with a cover-slip.   Add chloroform as evapora-
tion goes on.

In the middle of the nerve-fibre the pale granular
axis cylinder will be seen running through the swollen
and partially dissolved medullary sheath.

3.   Take another piece of fresh nerve ; tease it out on
a slide in a drop of osmic acid ·5 p. c., then place it
in a small quantity of the acid till it is black, covering
it up to prevent evaporation.

Wash it *thoroughly*, first with water, then with spirit,
and place it in hæmatoxylin till it has become well
stained.   Wash with water and tease out more com-
pletely in glycerine.   Observe

a.   The medullary sheath, stained black with the
osmic acid.

b.   The axis cylinder, stained with the hæmatoxylin.

c.   The nuclei of the primitive sheath seen at in-
tervals, also stained with hæmatoxylin.

d.   The break in the medullary sheath (**Ranvier's
node**) between each two nuclei.

4.   Stain with hæmatoxylin, or carmine, a short piece
of rather thick nerve (e.g. the sciatic of a mammal),
previously treated with ammonium bichromate and
alcohol.   Imbed, and cut as thin transverse sections
as possible ; treat these with carbolic acid &c., and
mount in Canada balsam.   Observe

a.   The cut ends of the nerve-fibres varying in
diameter, and in each

b.   The section of the stained axis cylinder sur-
rounded by

> c. A transparent ring indicating the former position
>   of the medullary sheath, which has been dis-
>   solved out in the process of mounting.
>
> d. The primitive sheath as a limiting circle.
>
> e. The arrangement of the fibres in bundles. The
>   perineurium between the fibres, and between the
>   bundles of fibres.

5. Open the abdomen of a recently killed frog, stretch
    the mesentery smoothly over a ring of cork, with a
    central hole of about one-third of an inch in diameter,
    and pin it in that position. Cut out from the body the
    piece so fixed to the cork, cutting away also the in-
    testine immediately connected with it.   Treat the
    piece still attached to the cork with gold chloride
    ·5 p. c. (Lesson V. B. § 4), taking care that the whole
    of the tissue is kept immersed in the fluid.

    Cut out a piece of the mesentery so stained, and
    mount it in glycerine. Trace out the nerves (coloured
    with the gold) towards the periphery.   Observe

> a. The division and branching of the nerves.
>
> b. The division of the nerve-fibres.
>
> c. The gradual loss of the medullary sheath.
>
> d. The continuation of the fine nerve-fibres, with
>   the nuclei of their primitive sheath very distinct,
>   into delicate threads, without a primitive sheath
>   and with frequent varicosities.

B. SYMPATHETIC NERVES.

    Cut out from the spleen of a large animal (e.g. ox) a
    small piece of one of the large sympathetic nerves
    running alongside the blood-vessels. Remove the loose
    connective-tissue sheath, and tease out the nerve care-
    fully in normal saline solution.   Note

    a.   The scanty medullated nerve-fibres.

    b.   The **non-medullated** nerve-fibres or fibres of Remak, constituting the bulk of the nerves: they are pale and granular, and having no medulla show distinctly the oval nuclei of the primitive sheath.

C.   SPINAL GANGLIA.

   1.   From the head of a frog, preserved in potassium bichromate, dissect out the Gasserian ganglion of one side (Hdb. p. 70.) Tease a portion of it, in a *very small* quantity of water or of glycerine, into small fragments. Take care that when the cover-slip is laid on, as little as possible escapes. Numerous **ganglionic corpuscles,** freed from their sheath, will be seen floating about, also others still surrounded by their sheaths and in position. Observe in the corpuscle

    a.   The granular protoplasmic **cell-substance** often containing pigment.

    b.   The large transparent **nucleus** with its conspicuous **nucleolus.**

    c.   The **sheath,** of connective tissue with numerous small nuclei.

    d.   Processes of the cell-substance. These, in most instances, will have been torn away in teasing.

   2.   Excise the ganglion on the posterior root of one of the spinal nerves of the frog. Mount it in a drop of potassium hydrate, and gently press on the cover-slip.

      Observe the now faintly visible ganglion-cells, arranged in groups, and the nerve-fibres passing over and among them.

3. Cut thin transverse and longitudinal sections of spinal ganglia (mammalian) previously hardened in chromic acid and stained in hæmatoxylin. Mount in glycerine or in Canada balsam; and observe

   a. The arrangement of the ganglionic corpuscles.

   b. The arrangement of the nerve-fibres.

   c. The connective tissue supporting both fibres and corpuscles.

   The nuclei, cell-substance, and sheaths of the corpuscles may also be very well seen.

D. SYMPATHETIC GANGLIA.

Lay open the abdomen of a frog, and carefully cut away the intestines. The abdominal aorta will be seen lying in the middle line, with the large spinal nerves on either side. Cut out the aorta and the nerves of both sides, *in one piece,* and place *flat* in gold chloride ·5 p. c. and treat as usual. Along the aorta and its branches, and on the sympathetic branches crossing from the spinal nerves towards the aorta, are numerous sympathetic ganglia. The whole piece may be searched with a low objective until the position of the smallest and least covered ganglia is ascertained. These can then be examined (in situ or after removal from the large piece) with a higher objective. Note

   a. The cell with granular, pigmented, protoplasm, nucleus and nucleolus.

   b. The sheath with its nuclei.

   c. The straight and the spiral processes of the cell-substance.

E.   SPINAL CORD.

1.   Cut a thin transverse section of the spinal cord of a
     mammal hardened in ammonium bichromate.   Stain
     with hæmatoxylin, treat with carbolic acid as usual,
     and mount in Canada balsam.   Observe

     a.   In the white substance, the cut ends of the
          nerve-fibres.   Compare with A, § 4.

     b.   In the grey substance (especially in the anterior
          cornua), the *multipolar ganglionic cells,* each
          possessing a nucleus with nucleolus, and proto-
          plasmic cell-substance, prolonged into branching
          processes.   The cell-substance often contains
          pigment.

     c.   The connective tissue support of the nerve-fibres
          and cells.

     (N.B.   The structure of the spinal cord will be more
          fully studied in a future Lesson).

2.   Cut a thin transverse section of the spinal cord of
     ox, preserved in potassium bichromate.   Tease out
     the section carefully, under a low power; the cells
     may thus be isolated.   Note

     a.   The nucleus, nucleolus, and protoplasmic cell-
     substance, the latter frequently containing a deposit
     of pigment.

     b.   The branched processes frequently broken short.

     c.   The undivided nerve-process about to form an
          axis cylinder.

     d.   The obscure fibrillation of this process and of
          the cell-protoplasm.

LESSON IX.

GENERAL PROPERTIES OF NERVOUS TISSUE. AUTOMATIC ACTIONS.

A. REFLEX ACTION.

1. Place on its belly a frog, which has been previously
    deprived of its brain by the demonstrator. Observe
    that its hind-limbs are drawn up under the body;
    but that it differs from the normal frog in the follow-
    ing respects.

    a. Its head is depressed, instead of being erect.

    b. Its fore-limbs are spread out, or flexed, instead of
        being held nearly vertical; thus the angle which
        the body makes with the table is diminished.

    c. There are no respiratory movements, either of
        the nostrils, or of the throat.

2. Gently pull out one of the hind-limbs, until it be-
    comes quite straight, and then let it go. It will be
    immediately drawn up into its old position under the
    body.

3. Gently tickle one flank with a feather or a blunt
    needle; a contraction of the flank muscles of that side
    will be observed.

4. Pinch the same spot rather sharply with a pair of forceps; the leg of the same side will be first extended, and then drawn up and swept over the flank; the movement tending to thrust away the points of the forceps.

5. Pinch with the forceps the skin round the anus; both legs will be drawn up and thrust out again; the movement tending as before to sweep away the points of the forceps.

Leave the animal alone for five minutes and watch it carefully: if no disturbing circumstances are brought to bear on it, it will remain perfectly motionless.

6. Place the animal on its back; it will make no effort to regain its normal position.

7. Pass a hook through the lower jaw, and fasten it to the cross-bar of a stand so that the body can be raised up and down. The hind-limbs, after a few movements of flexion and extension, will remain pendant and motionless.

8. Gently pinch the tip of one of the toes of either leg; that leg will immediately be drawn up.

9. Take two glasses, fill one with dilute sulphuric acid (·1 p. c.) and the other with water: lower the frog until the tip of one of the toes just touches the dilute sulphuric acid. In a short time the foot will be withdrawn. At once let the foot dip into the second glass containing water, in order to wash away the acid. Measure with a rapidly beating metronome, the time between the moment when the toe comes into contact with the acid and the moment when it is with-

drawn.   Make three such observations; and take the mean of the three.

10. Cut a small piece of blotting paper one or two mms. square, moisten it with strong acetic acid, and place it on the flank of the animal.   The leg of the same side will be speedily drawn up and swept over the flank as if to remove the piece of paper.

11. Place similar pieces of paper dipped in acetic acid on different parts of the body; different movements will be witnessed in consequence; all however tending to remove the irritating substance.

12. Wash off all the acid from the frog, and when it has become perfectly quiet, place it in a bason of water; it will sink to the bottom (unless the lungs be accidentally much distended with air), and no movements of any kind will be witnessed.

Observe that all the movements produced in the foregoing observations, although complicated, co-ordinated, and purposeful in character, are partial, and only by accident bring about locomotion.   However stimulated, the animal never springs or leaps forward.

In order that the same frog may serve for observations on the lymph hearts, B. I. § 1 should be performed here.

13. Make a small cut through the skin of the back[1], and with a fine glass tube inject one drop of a 1 p. c. solution of strychnia.   In a few minutes the slightest stimulus applied to any part of the animal will produce violent tetanic spasms of the whole body.

[1] Immediately underneath the skin of the back of the frog is the dorsal lymphatic sac, and any fluid placed in this rapidly makes its way into the blood.

L.   5

14. Through the opening at the back of the neck into the spinal canal made in destroying the brain, introduce a pithing needle or piece of stout wire, and destroy the whole of the spinal cord. Observe that the spasms immediately cease.

15. Repeat any of the above observations (§ 2—13). No reflex actions will now be produced.

B. AUTOMATIC ACTION.

I. The Lymph-Hearts.

1. Placing the animal again on its belly watch the movements of the posterior lymph-hearts. They may be seen beating on either side of the extremity of the urostyle, in a depression between that bone and the hip-joint. The contractions are generally visible through the skin, but become more evident if the skin be removed, care being taken not to injure the lymph-hearts themselves.

2. Observe that after destruction of the posterior part of the spinal cord the lymph-hearts cease to beat.

II. The Heart.

1. Lay the frog on its back, make a median incision through the skin, and from the middle of this make transverse incisions. Raising up the end of the sternum with a pair of forceps, cut it through a little above the end so as to avoid cutting the epigastric vein. Then again lifting up the sternum cut it through longitudinally, a little removed from the middle line, with a strong pair of scissors; pin back each part of the sternum, cutting through such muscles as may be necessary; the heart will be seen

in the pericardium beating with considerable regu-
larity and force.   Pinching up the pericardium with
a fine pair of forceps, open the pericardial cavity;
then tilt up the apex of the ventricle; a small band
of connective tissue will be observed passing from the
posterior surface of the ventricle to the adjoining
wall of the pericardium.   Seizing this band with the
forceps, divide it between the forceps and the peri-
cardial wall.   Lift up the apex of the ventricle, by
means of this band, and with a sharp pair of scissors
cut through the aortæ, the superior venæ cavæ, the
inferior vena cava, and the surrounding tissue.   Take
care not to injure the sinus venosus.   Place the heart
in a watch-glass, moistening it when necessary with
normal saline solution.   The beats will either not be
interrupted at all or for a very short time.

2.   Lifting up the apex of the ventricle by means of the
same band as before, cut through the ventricle with a
sharp pair of scissors at its upper third.   The lower
two-thirds of the ventricle will remain motionless
without any spontaneous beat; the upper third and
the auricles will continue to beat with regularity.

3.   By means of a longitudinal incision divide the
auricles with the attached portions of ventricle into
two lateral halves.   Each half will continue to beat.

III. **Cilia.**

Placing the frog on its back, cut through the lower
jaw, in the middle line, and carry the incision as far
as the point where the broad pharynx narrows into
the œsophagus, so as to expose as much as possible
the upper or dorsal wall of the throat.   Moisten the

5—2

mucous membrane, if it is at all dry, with normal saline solution, and place on it, between the two orbits, a small thin piece of cork. The cork will be seen to be driven by ciliary action down towards the œsophagus.

DEMONSTRATIONS.

The natural electric currents of nerves.

The negative variation attending a nervous impulse.

The phenomena of electrotonus.

Electrotonus as affecting irritability.

The law of contraction.

Measurement of the rapidity of the nervous impulse.

The transmission of nervous impulses in both directions.

The phenomena of urari poisoning.

The function of the anterior and posterior roots of the spinal nerves.

The peristaltic movements of the intestines and of the ureters.

LESSON X.

STRUCTURE AND PROPERTIES OF BLOOD-VESSELS.

A. THE LARGER ARTERIES.

1. Cut transverse and longitudinal sections of a small piece of a moderate sized artery imbedded in paraffin. Note

   a. The thin **inner coat** thrown into longitudinal folds by the contraction of

   b. The much thicker **middle coat**, consisting of alternating layers of elastic and circularly arranged muscular tissue. The disposition and relative amount of the elastic tissue vary much in different arteries.

   c. The **external coat** (tunica adventitia), consisting mainly of white fibrous connective tissue, with some elastic tissue not unfrequently arranged in layers, especially at the inner part of the coat. Add acetic acid to bring into view the nuclei of the muscular fibres.

2. Cut sections as above, and stain with picrocarmine, being careful to wash well with water before mounting. The fibrous connective tissue will be coloured pink, and the elastic and muscular tissue yellow.

3. Take as small an artery as possible, cut it open
    longitudinally, and pin it out on a thin piece of cork;
    immerse it for five or ten minutes in a ·5 p.c. solution
    of silver nitrate, then wash well with distilled water,
    and expose to light, also in distilled water, until it
    becomes of a brownish colour: mount it in Canada
    balsam, with the epithelium upwards. Observe the
    dark lines of the cement substance between the cells.
    If the artery has been left too long in the silver
    nitrate solution, or exposed too long·to light, silver
    will also be deposited in the substance of the cells.

4. Cut open longitudinally a large artery, e.g. carotid of
    sheep, from a freshly killed animal, and very gently
    scrape the internal surface. Tease out the material
    so obtained in normal saline solution.

    Observe the long, almost fusiform cells of the
    lining **epithelium,** with their somewhat oblong nuclei.
    Probably there will also be seen fragments of **fenes-
    trated membrane.** These will become obvious after
    the addition of acetic acid. The perforations in them
    are rendered more distinct by staining.

5. Tease out in water small portions of arteries pre-
    viously digested in artificial gastric juice. The tissue,
    after being well washed, will be found to consist
    almost entirely of **elastic laminæ.**

    Observe the gradations from an almost homogeneous
    elastic perforated membrane to a meshwork of elastic
    fibres.

6. From fresh arteries, and from arteries that have
    been preserved in potassium bichromate, tear off strips
    from the middle coat, and tease out thoroughly the
    pieces so obtained.

Note the bands of plain **muscular tissue** mingled with the elastic laminæ.

To the fresh specimens add acetic acid; the appearance of the rod-shaped nuclei will shew where the muscular element is present.

B. THE LARGER VEINS.

1. Repeat the above observations on the veins in place of the arteries. Relatively, the epithelial cells will be found to be broader, the elastic element to be less, the connective element to be greater, and the muscular element to be very variable.

2. Examine the structure and position of the valves in the veins of some large animal. Attempt to make fluid pass along them in the wrong direction, in order to shew the action of the valves.

C. CAPILLARIES AND SMALL BLOOD-VESSELS.

1. Tease out in glycerine the tail of a tadpole preserved in chromic acid, and either stained with hæmatoxylin or left unstained. Abundant capillaries will be seen. Note

    a. The outline of the channels.

    b. The nuclei on the walls, especially at the points of bifurcation.

    c. The branched connective-tissue corpuscles, in places partially surrounding the capillaries, and constituting the simplest form of the tunica adventitia.

    d. The connection of some of the processes of the connective-tissue corpuscles with the capillary walls.

    e. The blood-corpuscles within the capillaries.

2. Remove a small portion of pia mater from a perfectly fresh brain, and spread it out carefully in normal saline solution, washing it gently with the same, and mount.   Note

    a. The capillaries, with their nuclei and protoplasm bulging out into the channels.

    b. The small arteries, their adventitia, and the indications of their muscular coat composed of a single layer of fibres wrapped round the intima.

    c. The small veins generally filled with blood-corpuscles, with their muscular coat not so distinctly developed.

3. Gently irrigate with dilute acetic acid.   Note

    a. In the capillaries, the more distinct nuclei.

    b. In the small arteries,

        (*a*) The fusiform cells and nuclei of the adventitia, arranged longitudinally, with elastic fibres,

        (β) The nuclei of the muscular coat arranged transversely,

        (γ) The profile of the elastic intima,

        (δ) The oval nuclei of the epithelium arranged longitudinally.

    c. In the small veins, similar appearances to *b*, but less development of muscular nuclei.

4. Examine capillaries and small vessels previously injected with silver nitrate.
Note the pattern formed by the junction lines of the epithelium.

5.   Take a frog with a pale web, and introduce under
the skin of the back one drop of a 1 p.c. solution of
urari, and leave it under a glass jar till it makes no
motion on being pinched. It is of advantage that
this should not be the case in less than three-quarters
of an hour, otherwise the vessels will be abnormally
dilated.   Lay the frog on the frog-board, and tie not
too tightly a piece of soft cotton round the end of
the (e.g.) 3rd and 4th digits. Stick two pins into the
board a little distance from the hole ; by twining the
cotton round them the web may be stretched out
level above the hole.   Surround the web, and cover
the leg and body of the frog, with moist blotting
paper.   Put a *small* drop of water upon the web, and
cover it with a triangular piece of cover-slip, taking
care that the glass does not cut into the digits and
that no fluid flows over it.

Examine, first with objective 3, and then with 7. Note

a.   The course of the blood from the arteries to the
veins.   Sometimes a slight pulsation may be
observed in the arteries.

b.   In the small arteries and veins the rapid median
stream of red corpuscles, separated from the walls
of the vessels by the slower stream or inert layer,
in which the red corpuscles are rare.

c.   The white corpuscles in the inert layer, either
stationary and clinging to the walls of the vessel,
or moving slowly.

d.   The passage of corpuscles in single file through a
small capillary; the distension of the capillary
by the corpuscle.

e. The elasticity of the red corpuscles, observing the
way in which they bend and become deformed,
but regain their normal shape.

6. Remove the cover-slip, and soak up the fluid on the
web; touch the middle of the web with the tip
of a pointed glass rod that has been dipped in
an irritant, such as moderately strong sulphuric
acid, and replace the cover-slip. The early stages
of inflammation may be seen. Observe in suc-
cession

a. The dilatation of the arteries, the much more
distinct appearance of the capillaries, and the
enlargement of the veins.

b. A quickening of the current followed by a
slowing.

c. The crowding of white corpuscles in the capillaries
and veins, with a slowing of the current.

d. Stasis, or complete stopping of the current, with
a gradual obliteration of the outlines of the cor-
puscles.

e. The emigration of the white corpuscles.

f. The diapedesis of the red corpuscles from the
veins.

Notice that these effects are local, that they are of
greatest intensity in the spot touched, that they
extend for some distance round, but that the circula-
tion in the rest of the web is normal.

LESSON XI.

STRUCTURE AND ACTION OF THE HEART.

A. HEART OF SHEEP[1].

1. Observe the attachment of the parietal pericardium to the roots of the great vessels.

   Remember that the parts of the heart which are right and left in the body are called right and left after removal. The front of the heart may be recognized by a groove filled with fat, the interventricular sulcus, which runs from about the middle of the base of the ventricles to rather below the middle of the right margin of the heart. The front is also much more convex than the back. Holding the heart with the front towards you, note that the right ventricle, which will be on your left hand, is much more yielding than the left ventricle, which will be on your right hand. Note also the pulmonary artery arising from the middle of the heart at the upper part of the ventricles, and immediately behind this the aorta.

2. Tie a short glass tube into the superior vena cava and connect with it a piece of india-rubber tubing. Ligature the inferior vena cava and the left vena

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[1] The heart should be obtained from the butcher with the pericardium; to secure this it is advisable to purchase the lungs with the heart.

azygos which opens close beside it. Tie a glass tube about two feet in length into the pulmonary artery. Fill the india-rubber tubing with water, and squeezing it press the water onwards. The water will mount in the tube connected with the artery, and will only descend a little way on unclasping the india-rubber tubing. Pour water into the long glass tube by means of a funnel, and observe the column of water which the semilunar valves will sustain. Note the distension of the arterial walls and the bulging at the attachment of the valves. When the pressure is removed the artery by its elasticity returns to its previous dimensions.

3.   Repeat the above observation with the pulmonary veins and aorta.

4.   Compare the united sectional areas of the superior and inferior venæ cavæ when distended, with the area of the aorta below the origin of the innominate artery.

5.   Having removed the tubes, lay open the superior and inferior venæ cavæ, and bring the incisions to meet in the front of the auricle. Note

    a.   The size and form of the auricular cavity.

    b.   The auricular appendage with its muscular fretwork.

    c.   The **septum auricularum.**

    d.   The **fossa ovalis,** or expression of the fœtal foramen ovale, which is early closed by the growth of the septum auricularum.

    e. The **Eustachian valve** immediately beneath the entrance of the inferior vena cava, and again beneath this,

    f. The opening of the comparatively large **left azygos vein.**

    g. The auriculo-ventricular orifice.

6. Cut open longitudinally the azygos vein, and observe the **coronary vein** opening into it close to the heart.

7. Cut away most of the auricle, and holding the ventricle in the left hand, pour water suddenly into the auriculo-ventricular orifice. The valves will float up and close. Note the star-shaped junction.

8. Introduce a pair of scissors between two of the valves, and cut through the wall towards the apex. Having arrived at the bottom of the ventricular cavity, turn the scissors sharp round and carry an incision at an acute angle with the previous one, alongside the septum, towards, but not into, the pulmonary artery. Lifting up the flap, note

    a. The **tricuspid valves,** their form, attachment to the auriculo-ventricular ring, the **chordæ tendineæ,** and papillary muscles.

    b. The thickness of the ventricular wall, the columnæ carneæ.

    c. The projection of the septum at the apex.

9. Holding the heart vertically, pour water into the pulmonary artery; observe from below the form of the semilunar valves, and their mode of closing.

10. To observe the valves from above, insert into the pulmonary artery a short wide tube, fill it with

water, and cover it with a piece of glass, excluding air-bubbles.

11. Prolong the incision of § 8 so as to lay open the pulmonary artery.   Note

    a.   The form and attachment of the semilunar valves.

    b.   The small nodule in the middle of the free edge of each valve, the **corpus Arantii.**

    c.   The slight depressions in the arterial walls opposite each valve, the **sinuses** of **Valsalva.**

12. Lay open the left auricle in a manner similar to that employed for the right.   Study as on the right side the action of the left auriculo-ventricular valve (the mitral).

13. Lay open the left ventricle in a manner similar to that employed on the right side, carrying the incision at first along the extreme left of the heart.   Note the thick walls, the mitral valve, &c.

14. Lay open the aorta, and examine its semilunar valves, corpora Arantii, and the sinuses of Valsalva, which are here very distinct.   Note that the coronary arteries open respectively into the two anterior sinuses.

B.   HEART OF FROG.

1. Expose the heart of a pithed frog in the manner directed in Lesson IX. B. 11.   With the pericardium intact, observe the pulsations of the heart, noting the alternate beats of the auricles and the ventricle; and the synchronous beats of the two auricles.

2. Lay open the pericardium and observe

    a.   The synchronous contractions of the two auricles, followed almost immediately by

    b.   The contraction of the ventricle, note that the ventricle during its contraction or systole becomes pale and conical, and that its apex is thrown forwards and upwards.

    c.   The slight contraction of the bulbus arteriosus immediately succeeding the ventricular systole.

    d.   The pause, or diastole, which follows before the auricle again beats.   .

    e.   The increased redness and distension of the ventricle after the auricular, and immediately preceding its own systole.

3.   Divide the band attaching the ventricle to the posterior pericardial wall, and turn the apex of the ventricle over.   Observe

    a.   The junction of the two superior venæ cavæ with the inferior vena cava to form the sinus venosus.

    b.   The white line marking the junction of the sinus venosus with the right auricle.

    c.   The cardiac branches of the pneumogastric, running along each superior vena cava and then plunging into the interior of the heart.

    d.   The series of pulsations beginning with the venæ cavæ, superior and inferior, next the sinus venosus, then the auricles, then the ventricle, and finally the bulbus arteriosus.

4.   Place a light lever on the heart, and bring the end of the lever armed with a bristle, to mark on the revolving drum.   Take a tracing of ventricular pulsations with the drum at a moderate speed.

Note the rise and fall of the lever, indicating the change of form during contraction. The rise increases

at first rapidly, then more slowly, to a maximum, and the fall is similarly at first slight, then more rapid, but finally slow again.

5. Pass an interrupted current through the pneumo-gastric nerve.

  a.   Observe during and for a short time after the passage of the current, the heart remains with all parts in diastole.

  b.   The period of rest (inhibition) is followed by a period (reaction) in which the beats are quicker and more forcible ; and then the previous normal condition is regained.

6. Take a tracing of the above on the drum, indicating on the curve the moment at which stimulation was begun, and ended.

7. Cut across the aorta, to enable the heart to empty itself of blood. Pass a silk ligature underneath the bulbus arteriosus and two underneath the inferior vena cava. Tie them *loosely*, ready to be tightened at any moment. Raise up the wall of the inferior vena cava with a fine pair of forceps, as far from the heart as possible, consistently with making an incision into the vein near the liver. Make a V-shaped incision, and introduce into this a cannula as large as can be conveniently used. Tie the tube in the vein by means of the lower of the two ligatures. Fill the tube with normal saline solution, and by blowing gently, wash all blood out of the heart. Then partly fill the tube with a ·5 p.c. solution of gold chloride. Inject this in the same manner, until the solution begins to issue from the aorta. Then ligature the bulbus arteriosus. Again inject, and whilst the

heart is distended tie the remaining ligature round the inferior cava just beyond the end of the cannula. Immerse the whole heart in gold chloride solution. In a few minutes it will become rigid. Lay open the auricular wall on each side. Observe the distinct cavities of the auricles. Cut out the septum, expose it to light and subsequently examine in glycerine. The branches of the pneumogastric and the ganglia with spiral cells may be seen in it. Examine in the same way the wall of the sinus venosus along which the cardiac branches of the pneumogastric were seen to run. Numerous ganglia are scattered over these.

8. Wash the ventricle thoroughly, and when, after exposure to the light, it has become stained, transfer to spirit and then to absolute alcohol, as directed in Lesson I. § 13. When sufficiently hardened, prepare transverse sections of it. Observe the interlacement of the bundles of fibres. The walls are of a distinctly spongy character, the small central cavity being continuous with a labyrinth of small passages. In this way the blood is brought into direct contact with the muscular fibres, though no capillaries or blood-vessels are present.

Note the absence of ganglia in all parts but the extreme base.

DEMONSTRATIONS.

Experiment of Stannius.

The action of the mammalian heart.

Pneumogastric inhibition in the mammal.

Sounds of the heart.

Endocardial pressure of frog.

LESSON XII.

BLOOD PRESSURE.

A. MINOR ARTERIAL SCHEME[1].

1. Clamp the india-rubber tube at its proximal end close to the pump, and leave the glass tube open so that all the water flows through the latter. Work the pump with a uniform force at about 30 to 40 beats a minute. To ensure regularity, the strokes had better be timed with a metronome. The water will flow from the open mouth of the glass tube in jerks, corresponding to the beats of the pump. At each stroke as much will issue from the distal end as enters at the proximal end. ·

2. Introduce into the open mouth of the glass tube a fine nozzle, so as to offer considerable resistance to the outflow of fluid. Work the pump with the same force and frequency as before. The outflow will still be intermittent, though less fluid will issue from, and consequently less enter into, the tube at each stroke.

3. Clamp the proximal end of the glass tube and unclamp the elastic tube. Let the distal end of the latter be quite open. Work the pump as before. There being little resistance to the outflow, the elasticity of the tube is not called into play, and consequently the flow will be, as in the case of the glass tube, intermittent.

[1] See Appendix.

4. Working the pump as before, insert the fine nozzle
into the open mouth of the tube. Considerable resist-
ance will now be offered to the outflow of fluid, the
elasticity of the walls of the tube will be called into
play, and the water will issue from the end of the
tube in a continuous instead of an intermittent
stream. If the tube be sufficiently long and suffi-
ciently elastic, in proportion to the force and fre-
quency of the strokes, the flow will be absolutely
continuous.

B. MAJOR ARTERIAL SCHEME[1].

The pump represents the heart; the small tubes re-
present the resistance of the small arteries and capil-
laries. The tubes on the proximal side of this resistance
represent the arteries, those on the distal side the veins.

1. **The Mercurial Manometer.**

The manometer A is connected with the arterial, V
with the venous tubes.

a. Open the clamps marked C, C' and C", so that as
little resistance as possible intervenes between
the arterial and venous tubes. Bring the mano-
meters to mark on the revolving cylinder, placing
V about an inch under A, *in the same vertical
line.* Work the pump steadily, regulating the
time with the metronome.

In A, the mercury rises at each stroke, and in
the interval between each two strokes falls
again to its previous level. (The momentum of
the mercury frequently carries it below this level,
and the descent may be followed by one or more
oscillations.)

[1] See Appendix.

6—2

In V, a similar rise and fall is observed, of nearly if not quite the same extent.

b. Close the clamps C, C′ and C″, so that the capillary resistance becomes very considerable.

In A, the mercury rises rapidly at the first stroke and at the end of the stroke begins to fall again, but more slowly than was the case in *a*. It has not fallen far before the second stroke raises it to a higher level than before. On falling still again, it is once more raised to a yet higher level, but the increase is not so great as before. Each succeeding stroke has a similar effect. Thus at the end of a few strokes, *the mean arterial pressure* is reached, with comparatively small oscillations corresponding to the strokes of the pump.

On the strokes ceasing, the mercury *gradually* falls until the previous level is reached.

In V the mercury rises to a much less extent than was the case in *a*, a slight mean pressure much less than in A is established, marked either with no oscillations at all or such as are much less conspicuous than those of A.

Owing to the presence of the resistance, a mean pressure (arterial blood pressure) is established on the proximal (arterial) side of the resistance. This pressure is marked by oscillations synchronous with the strokes of the pump. On the distal (venous) side the mean pressure is much less and the oscillations are either slight or altogether absent.

2. **Flow from Arteries and Veins.**

Remove the clamps from the fine nozzles *a* and *v*. Let the clamps C, C′ and C″ remain closed. Set the

pump going. The flow from *a* on the proximal (arterial) side is in jets; that from *v* (venous) side is uninterrupted or nearly so.

3. Sphygmograph.

Bring the levers A (arterial side) and V (venous side) to write on the revolving drum, one under the other.

a.   Open the clamps C, C′ and C″, and set the pump going. The two levers describe two nearly straight lines, a slight rise only being evident (and that to about the same extent in both) at each stroke.

When there is little or no resistance in the capillaries, comparatively little distension of the arterial walls is produced at each stroke of the pump.

b.   Close the clamps C, C′ and C″.

The lever A now describes a well-marked curve with each stroke of the pump.

Observe the sudden rise to a maximum, the commencing fall, the break in the fall, followed by a slight rise (dicrotic wave) and the final descent.

The lever V describes now a straight line.

The rise in pressure at each stroke indicated by the mercurial manometer is accompanied by a distension of the proximal (arterial) part of the tubing, indicated by the rise of the lever. This is the pulse.
On the distal (venous) side of the resistance no pulse is visible.

4. Progression of the Pulse-wave.

Place two levers, one A, as near as possible to the pump, the other A′, as near as possible to the re-

sistance. Bring the two levers to mark on the cy-linder *the one exactly beneath the other.*

(The pressure exerted by the two levers must be as nearly equal as possible.)

Observe that each rise of A begins a little before, and is over a little before that of A'.   In other words, the pulse of A' is a little later than that of A.

(By means of a tuning-fork this interval may be measured, and the length of tubing between the two levers being known, the rate of progression of the pulse wave ascertained.)

5.   While the pump is working, the clamps being closed and the manometers tracing their curves, *gradually* diminish the resistance by opening slowly first C' and then C''.

The arterial pressure curve will gradually fall, still marked by the pulse oscillations; the venous curve will gradually rise.

Diminution of capillary resistance lowers arterial, but in-creases venous pressure.

6.   Close the clamps C' and C'', and take tracings with the manometers, then gradually reduce the strength of the strokes of the pump.

Both arterial and venous pressure will diminish.

7.   The clamp C being closed, the main arterial trunk of the scheme divides into two chief branches, X and Y, each with its own resistance and venous tube.

    a.   Work the pump with great regularity, and mea-sure the quantity of fluid which escapes during a given time (say ten seconds) from the venous tube of X, and from that of Y, by the side tubes *x* and *y.*

b. The clamp of X remaining closed, open that of Y, and the pump working exactly as before, measure again the outflow during ten seconds.

The outflow of Y will be increased. That of X on the other hand will be diminished, though the resistance in X is the same as before.

The flow of blood through an artery is dependent not only on the resistance offered by its own small arteries and capillaries but also on that of other arteries.

DEMONSTRATIONS.

The effects in the rabbit on the temperature of the ear, and on the calibre of its blood-vessels, following

a. Stimulation of the central end of the great auricular nerve.

b. Section of the sympathetic nerve in the neck.

c. Stimulation of the peripheral end of the sympathetic.

Normal kymographic tracings of the blood-pressure of a mammal obtained by the use of a mercurial manometer.

The effects on the arterial blood-pressure, as indicated by the tracing, produced by

a. Inhibition of the heart through stimulation of the peripheral end of the vagus.

b. Dilatation of the small blood-vessels through stimulation of the central end of the depressor nerve.

Methods of measuring the velocity of the blood-current in large vessels.

Comparison of venous and arterial pressure.

Use of the sphygmograph.

Use of the cardiograph.

LESSON XIII.

STOMACH AND SALIVARY GLANDS.

STRUCTURE OF THE STOMACH.

1. MAKE transverse vertical sections from the middle or cardiac end of a cat's or a dog's stomach which has been hardened in potassium bichromate. Stain some of the sections with hæmatoxylin or carmine, leave others unstained; mount them in glycerine. The general features will best be made out with a low power. Observe

    a. Externally, the connective tissue layer of the peritoneum.

    b. The **muscular coat**, consisting of an outer longitudinal and an inner circular layer of unstriped muscle. If the sections are accurately transverse, the former will appear as a cross section of a number of bundles with connective tissue running in between them from the peritoneum, the latter as a continuous layer. On the inner side of this may also be seen a much thinner oblique muscular layer.

    c. The **submucous coat** of connective tissue.

    d. The **muscularis mucosæ**, divided more or less distinctly into an outer longitudinal and an inner circular layer.

e.   The **mucous coat.**   Note in this

a.   The gastric glands with their openings and the ridges between the openings. Try to make out the bifurcation of some of the glands.

β.   The fine connective tissue immediately internal to the muscularis mucosæ, surrounding the bases of the glands, and sending up processes between them. Towards the surface the fibres have a much closer arrangement and appear as a number of slender, comparatively dark, bands, which stain deeply : be careful not to confound these with the mouths of the glands. Mark the scarcity of leucocytes.

γ.   Using a high power, note further the clear slender **columnar** epithelium lining the upper portions of the glands and covering the free surface of the gastric mucous membrane forming the ridges between the glands.

δ.   The large coarsely granular **ovoid**[1], or 'peptic' cells, placed in the middle and lower part of each gland. They frequently cause a bulging outwards of the basement membrane, especially during digestion.

ε.   The small pale and finely granular **central** cells, cubical or polyhedral in form, intermixed with the ovoid cells, and best seen at the lower part of the gland.

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[1] Since the functional importance of the different kinds of cells is as yet very uncertain, it is thought best to give them names which do not imply any special function.

The columnar cells will stain little or not at all with hæmatoxylin, the central cells moderately, and the ovoid cells deeply, more especially their nuclei.   The relative amount of colouration, however, depends very much upon the state of nutrition of the cells.

2. ⸰ Make longitudinal vertical sections from the same part of the stomach.   Note the difference in appearance of the muscular layers; in other respects the sections resemble the transverse ones.

3.   Make transverse and longitudinal vertical sections from the pyloric end of the stomach, staining them with hæmatoxylin, and note

   a.   The greater thickness of the circular muscular layer, and the absence of the oblique layer.

   b.   The more frequent branching of the glands, and the absence of ovoid cells.   The ovoid cells do not suddenly disappear as the pylorus is approached, so that glands containing a few such cells may be seen.

4.   Prepare surface sections of a cat's or dog's gastric mucous membrane, which has been taken warm from the body and placed in absolute alcohol, cutting across the glands; stain some, leave others unstained. Observe

   In sections of the upper part of the gland,

   a.   The small central lumen.

   b.   The basement membrane.

   c.   The layer of small deeply-stained cells immediately underneath the basement membrane.

   d.   The columnar cells with their broad ends abutting on the lumen.

In sections of the lower part of the gland,

a.   The small irregular lumen, often completely closed up.

b.   The basement membrane.

c.   The central cells surrounding the lumen.

d.   The ovoid cells between the central cells and the basement membrane.

5.   Gently tease out a section.   In this way some of the glands will be isolated, and their bifurcating or even branched ends, with the arrangement of the cells, readily observed.

STRUCTURE OF THE ŒSOPHAGUS.

1.   Make transverse vertical sections from the lower third of a rabbit's œsophagus, and compare them with the corresponding sections of the stomach.   Note the following points of contrast :

a.   The external muscular layers contain striped as well as unstriped muscular fibres ; sections from the upper part of the œsophagus have no unstriped fibres.

b.   The submucous tissue contains *mucous glands.* Each of these consists of a duct, dividing and ending in dilatations, the alveoli.

The ducts are lined with columnar epithelium, and the alveoli with spheroidal or cubical cells flattened against one another.

c.   Traces of the muscularis mucosæ.

d.   The papillæ of the mucous membrane.

    e.   The epithelium forming a layer several cells deep, the deeper being columnar or spheroidal, the superficial cells flattened (cf. Epidermis, Lesson XXII.).

STRUCTURE OF THE SALIVARY GLANDS.

1.   Prepare sections of mammalian salivary gland which has been removed warm from the body, cut up into small pieces, and placed in ammonium bichromate for a few days, or straight into absolute alcohol. Stain some with hæmatoxylin or carmine, leave others unstained.

With a low power observe

    a.   The division of the section into irregular angular areas, by connective tissue septa, which, if the section includes the circumference, will be seen to proceed from the sheath of the gland. These are the primary **lobules**; they may be seen to be divided into smaller lobules, but probably not distinctly.

    b.   The **alveoli**, appearing with objective No. 3 or 4 as small round bodies closely aggregated together to form the lobules; each will be seen to consist, more or less clearly, of a group of cells surrounded by connective tissue, continuous with that of the septa.

    c.   At intervals oblique and transverse sections of the small **ducts.** They are usually stained darker than the alveoli, are not surrounded by a sharp ring of connective tissue, and have a well-defined lumen.

2.   With a high power observe that

    a.   The alveoli vary considerably in size, and fre-
quently have no lumen; when visible the lumen
is an irregular central space between the cells.

    b.   The alveolar cells are comparatively large and
as a rule globular, but modified in form by being
pressed together; each contains a round nucleus
situated generally in the outer part of the cell.

    c.   The epithelium of the small ducts consists of a
single row of slender columnar cells, the inner
borders of which apparently coalesce and form a
distinct ring bounding the lumen: there is no
such distinct boundary to the outer (circum-
ferential) part of the cell, which especially in
hæmatoxylin specimens has a well-marked stria-
tion. Each cell contains an oval nucleus, staining
deeply, situated in the inner part of the cell.

3.   Cut sections of the pancreas of a dog, and observe
that its structure is fundamentally the same as that
of the salivary glands.

LESSON XIV.

SALIVA AND GASTRIC JUICE.

SALIVA.

1. INDUCE the secretion of saliva by chewing a small piece of india-rubber tubing, by filling the mouth with ether vapour, or by placing a crystal of tartaric acid on the tongue.

   Allow the mixed saliva to stand until the turbidity has settled down into a sediment. To a portion of the clear fluid, add acetic acid; a thick stringy deposit of **mucin** will take place, which does not dissolve in excess of acid.

2. Filter off the mucin; to the clear filtrate add a few drops of a saturated solution of potassium ferrocyanide. The turbidity which results indicates the total quantity of albuminous bodies present (cp. Lesson III. § 10 c).

3. Take three test-tubes, A, B, C, and place in each the same quantity of starch mucilage[1]. Dilute a little fresh saliva with five times its volume of water, and divide it into two parts. Add one part to B.

[1] To prepare starch mucilage, take a little wheaten starch, and rub it into a thin paste with cold water. Pour it into a beaker containing about two hundred times its volume of boiling water, boil for about five minutes and let it stand. Decant the clear supernatant fluid.

Thoroughly boil the other part for three or four minutes, and add it to C. Then place the test-tubes, A containing starch paste only, in the water bath at a temperature of $35°$ C. for ten minutes.

Divide the fluid in A into two parts.

a. Add a drop or two of a solution of iodine; the characteristic blue colour produced by starch and iodine will appear.

b. Add a few drops of Fehling's fluid, just sufficient to give the mixture a blue tinge, and boil; no discolouration or deposit will take place, shewing the absence of sugar.

Treat the fluid in B in the same way.

a. There will either be no blue colouration or but a slight one, shewing the disappearance or diminution of starch. There may be a violet colour due to the presence of dextrine.

b. There will be a copious yellow precipitate, due to the reduction of cupric to cuprous oxide, shewing that sugar has replaced starch. Take a little grape-sugar, and boil with Fehling's fluid, to observe the normal effects.

Treat the fluid in C in the same way.

Its behaviour as regards both *a* and *b* will be exactly the same as that of A, unless there happen to be an appreciable quantity of proteids present in the saliva, in which case the blue colour may be changed to violet (cp. Lesson III. § 11 *c*).

A shews that the mucilage does contain starch and does not contain any grape-sugar.

B shews that under the action of the saliva the starch disappears and grape-sugar appears in its stead.

C shews in the first place that the saliva itself contains no grape-sugar, and in the second place that the activity of the saliva is destroyed by exposure to the temperature of 100° C. This destruction by heat is one of the proofs that the activity of saliva is due to the presence in it of a *ferment*—the so-called **ptyalin.**

4. Take three test-tubes. Pour into each the same quantity of saliva and of starch. The quantity of saliva should be small; two or three drops of a diluted saliva is sufficient. Keep A at 35° C., B at the ordinary temperature, C surrounded by ice and salt. Examine portions from each at intervals, with iodine and Fehling's fluid.

The starch in A will be rapidly converted into sugar; that in B much more slowly, and in C hardly at all. Then expose B and C to 35° C.; the conversion will be rapid and complete.

This shews that the action of the saliva is favoured by a temperature as high as 35°, retarded at a lower temperature, and arrested without any *destruction* of the ferment at about the temperature of 0° C.

5. Pour saliva, diluted with five times its bulk of water, over raw starch[1], and expose it to 35° C., shaking frequently. The conversion will be extremely slow; the water above the starch will not contain sugar for an hour or two.

6. Place some dilute starch mucilage in one dialyser[2] (A), and another portion with saliva in another (B).

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[1] Arrow-root will do very well.

[2] A dialyser can easily be made by tightly tying a piece of parchment paper over the mouth of a test tube and cutting off the closed end.

Leave them for twenty-four hours. Then examine the external water in each. That from (*A*), tested with iodine and Fehling's fluid, will give no trace of starch or sugar. That from (*B*) will contain sugar, but no starch. Sugar dialyses, but starch does not.

7. Look at a little fresh saliva under the microscope with a high power. Disregarding the flat epithelial cells from the mucous membrane of the mouth, note the so-called salivary corpuscles, which are larger than the ordinary white blood-corpuscles, but in other respects indistinguishable from them. In many, a very active Brownian movement of the granules within the corpuscle may be observed.

<div align="center">GASTRIC JUICE.</div>

1. **Artificial Gastric Juice.**

    a. Remove the mucous membrane from the middle region of a pig's stomach. Mince it finely. Put it in a flask with two hundred times its bulk of hydrochloric acid ·2 p. c., and place the flask in the warm chamber at a temperature of 35°C. After some hours nearly the whole will be dissolved. Decant, and filter the decanted fluid. A solution of pepsin in hydrochloric acid will be obtained; it will, however, contain a considerable quantity of peptones.

    b. Cut a portion of the same mucous membrane into minute pieces. Remove with filtering paper as much moisture as possible. Cover the pieces with strong glycerine. After a few hours the glycerine will become strongly impregnated with pepsin. It may be kept for a great length of

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time, and the glycerine may be several times
renewed. A few drops of this glycerine extract
added to 100 cc. of hydrochloric acid ·2 p. c.
will yield a fluid of high digestive power.

2. **Action of Gastric Juice.**

a.   Take four test-tubes. In A place 5 cc. of hydro-
chloric acid ·2 p. c. In B 5 cc. of artificial gas-
tric juice. In C 5 cc. of the same juice, care-
fully neutralized. In D 5 cc. of the same juice,
thoroughly boiled. Put the same quantity of
fibrin[1] in each, and expose in the warm chamber.
Examine after a few hours.

A, the fibrin will be swollen and transparent, but
not dissolved; on neutralization it will appear
unaltered.

B, the fibrin will be wholly digested.

C, the fibrin will be opaque and unaltered.

D, the fibrin will be like that in A.

These experiments shew that acid alone (A)
and pepsin alone (C) will not digest, and that
pepsin loses its power on being heated to boiling
point (D). Now add acid again to C, and place
it in the warm chamber. Digestion will take
place. The neutralization has only suspended,
not destroyed, the action of the pepsin.

b.   Take two test-tubes, with 5 cc. of gastric juice
and a morsel of fibrin in each.

Place A in the warm chamber. Surround B with
ice, or put it in a cold spot.

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[1] The fibrin should be previously boiled, in order to destroy the small
quantity of pepsin frequently present.

The fibrin in *A* will be digested rapidly; that in *B* very little or not at all.

c.　To 5 cc. of gastric juice add a small piece of fibrin and keep it in the warm chamber. When digested throw in another flake of fibrin. Continue this as long as the fibrin disappears.

When digestion appears to cease, add 10 cc. of hydrochloric acid ·2 p. c. Digestion will begin again.

It was arrested partly by the presence of concentrated peptones, and partly by the consumption of acid. Fresh acid and dilution sets it going again.

3.　Products of Gastric Digestion.

a.　To 20 cc. of serum or egg-albumin add 20 cc. of ·2 p. c. hydrochloric acid. Place it in the warm chamber. From time to time remove a few cc. and boil. The first specimens will coagulate as usual, but the coagulation becomes less and less until at last there is not even a turbidity on boiling.

When this stage has been reached, carefully neutralize the remainder. A copious precipitate occurs, which, separated by filtration, is soluble in dilute acids and alkalis. This is *acid albumin*, or *syntonin*, or *parapeptone*. (Cp. Lesson VII. § 13.) If the neutralization has been perfect, the filtrate from the precipitate contains no albumin; the whole of the serum or egg-albumin has by the action of the acid become converted into acid albumin.

b.　Digest a handful of fibrin or other proteid with artificial gastric juice. Decant the fluid,

7—2

filter through a coarse filter; and carefully neu-
tralize it; a deposit of parapeptone, variable in
amount, will take place. Filter off the parapep-
tone. The filtrate contains peptones.

4. Determine the following *characters of peptones.*

   a.   Their solutions are not coagulated by boiling.

   b.   They are not precipitated by nitric acid.

   c.   They are precipitated by mercuric chloride.

   d.   They give Millon's and the xanthoproteic re-
          action.

   e.   With a very small quantity of Fehling's fluid
          they give a precipitate which dissolves in excess,
          forming a *red* and then a violet solution.

   f.   They dialyse readily. Pour on one dialyser (A)
          a solution of peptones, on another (B) egg-
          albumin. In a short time the external water of
          A will give evidences of proteids, but not that
          of B.

4. **Action of Gastric Juice on Milk.**

Prepare some artificial gastric juice either as in § 1 *a*,
or by simply rubbing up the mucous membrane of
any mammal with a little warm water, and filtering.

   a.   Pour into a test-tube 5 cc. of fresh milk and add
          to it a little of the artificial gastric juice; there
          will be a flocculent precipitate varying in degree
          with the amount of acid in the juice. Place it
          in a warm chamber and observe from time to
          time. The precipitation will become more de-
          cided, so that in two or three hours there is a
          clot floating in a clear fluid. This is due to

the casein of the milk being precipitated by the gastric juice, and carrying down with it the fat-globules. If it be left in the warm chamber till the next day, the fluid will be found to be again 'milky' and to contain peptones; the gastric juice has acted on the precipitated casein, turning it first into para-peptone and then into peptones, and so setting the fat-globules once more free.

b. Neutralize a little gastric juice carefully with sodium carbonate, add it to 5 cc. of milk, and place it in the warm chamber. Except that there is no precipitation on mixing the fluids, the results are the same, shewing that the clotting of the milk is not due to the *acid* of the juice.

The following experiments may be made on **Milk.**

1. Examine a drop of fresh cow's milk under the microscope with objective no. 7. Note that it consists of a clear fluid containing a large number of highly refractive **(fat)** globules of varying size. Add a drop of acetic acid; in a short time the globules in contact with one another will coalesce. The acetic acid dissolves the thin envelope of casein or alkali albuminate which prevented them running together.

2. Test the reaction of fresh cow's milk with litmus paper. It will be found to be **alkaline :** occasionally it is acid owing to the presence of free lactic acid.

   Place about twenty cubic centimetres in a warm place for forty-eight hours; then again test the reaction, it will be found to be acid; this is due to **fermentation,** in the process of which the **milk-sugar** is converted into lactic acid.

3. Neutralize a little milk with dilute acetic acid; no
    precipitate will fall. Continue to add the acetic
    acid *carefully*, warming to 35° C.; a precipitate of
    casein will occur, carrying down with it nearly all
    the fat. When there is a distinct precipitate, no
    more acetic acid should be added, as casein is soluble,
    though not very readily, in excess; it is not pre-
    cipitated on merely neutralizing, since there are
    alkaline phosphates present in milk. To precipitate
    the whole of the casein, the milk must be very much
    diluted.

4. Filter the milk from which the casein and fat have
    been precipitated, and test the clear filtrate for
    albumin (Lesson III. § 10); a small quantity will be
    found.

LESSON XV.

INTESTINE. BILE AND PANCREATIC JUICE.

STRUCTURE OF INTESTINE.

THE outer coats of the intestine have the same general characters as those of the stomach (Lesson XIII. § 1, *a. b. c. d.*), except that there is no oblique muscular layer.

1. Prepare vertical sections of a cat's or dog's **ileum** hardened in chromic acid. Stain some with hæmatoxylin, leave others unstained, and mount in glycerine. Observe in the *mucous coat*

A. The conical projections of the mucous membrane, or **villi,** either extended and long, or contracted and short with the surface thrown into folds. Note

    a. The epithelium, consisting of **conical** cells, each with a hyaline or striated border, granular proto-. plasm and oval nucleus ; the free borders of the cells frequently appear to have coalesced into a narrow highly refractive band.

    b. The **goblet** cells, irregularly scattered among the former, sometimes abundant, sometimes scanty or absent ; they are seen in the section as transparent round or oval cells with sharp outlines.

    c. The **adenoid** tissue, forming the substance of the villus : this consists of a fine meshwork of fibres

with nuclear-like thickenings at the nodal points. The meshes are seen to be crowded with leucocytes.

There may also be seen with varying distinctness

d. Beneath the epithelium indications of capillary blood-vessels in the form of rows of blood-corpuscles, and sometimes the outlines of the walls with their nuclei.

e. If the intestine be that of an animal killed during digestion, the 'lacteal radicle' in the centre of the villus as a club-shaped mass of finely granular matter.

f. Unstriped muscular fibres as narrow bands running up the villus.

B. The depressions of the mucous membrane or **glands of Lieberkühn.** Note that

a. The epithelium lining them consists of short columnar cells. Observe their graduation into the cells covering the villi.

b. There is a distinct basement membrane immediately beneath the epithelium. This is formed of connective-tissue corpuscles very much flattened and fused together into a membranous sac; the outlines of the cells are not seen in the section, but their nuclei are fairly conspicuous.

c. The lumina of the glands are small, but in thin sections distinct.

C. The adenoid tissue around the bases of the glands of Lieberkühn and between them and the muscularis mucosæ. This, unlike the corresponding tissue in the

stomach (Lesson XIII. § 1. *e. β.*), has a large number of leucocytes in its meshes.

D. The **closed follicles**, isolated or aggregated into **Peyer's patches**; the follicles are round or oval masses of adenoid tissue crowded with leucocytes, lying immediately beneath the surface epithelium and often stretching down into the submucous tissue. They are in the midst of the glands of Lieberkühn, and the villi are absent over them. They will be more fully studied under Lymphatics (Lesson XVI. §§ 1. 2. 3).

2. Tease out in water a vertical section from the ileum or jejunum, and observe more closely,

    a. The individual characters of the conical cells covering the villi, their often branched central process, and the hyaline peripheral border.

    b. The goblet-cells; they will be seen to be flask-shaped cells with the greater part of the protoplasm replaced by a cavity filled with granules (of mucus) or clear fluid, a small portion only being left around a generally misshapen nucleus at the base of the cell. In the older goblet-cells may be seen a round opening at the top through which the mucus has been discharged.

       The characters of these cells will be still better seen by teasing out a small fragment of intestinal mucous membrane which has been treated with osmic acid.

    c. The characters of the cells and the basement-membrane of the glands of Lieberkühn.

3. Prepare sections of the ileum or jejunum through the glands of Lieberkühn parallel to the surface, and compare them with the vertical sections (§ 1, B. C.).

4.   Prepare vertical sections of the duodenum at its com-
     mencement close to the pylorus. It will be seen that
     the villi and glands of Lieberkühn are present as in
     the rest of the small intestine, but that the closed
     follicles are scanty or absent.   Note further

     The **glands of Brunner.** Each has a duct with base-
     ment-membrane, columnar epithelium and distinct
     lumen.   This dipping down into the submucous tissue
     forms a terminal knot of convoluted and anastomosing
     tubes, so that it has something of the appearance of
     a lobule of the salivary gland; it is, however, struc-
     turally more allied to a sweat-gland (Lesson XXII. § 2).

5.   Snip off a few villi from a perfectly fresh intestine of a
     mammal, and examine in normal saline solution.

     a.   Observe the epithelium *en face*, and the characters
          of the individual cells.

     b.   Irrigate with dilute acetic acid; observe the nuclei
          of the plain muscular fibres.

6.   Prepare vertical transverse sections of the **large in-
     testine.**   Observe

     a.   The longitudinal muscular coat, thin except where
          the section has passed through one of the three
          conspicuous bands.

     b.   The circular coat, thick and well developed.

     c.   The mucous membrane, frequently thrown into
          longitudinal ridges.

     d.   The entire absence of villi.

     e.   The glands of Lieberkühn; they are larger than in
          the rest of the intestine, and their features, through
          the absence of the villi, much more easily seen.
          The epithelium covering the free intestinal sur-

face or the ridges between the glands, has the same
slender columnar character as that covering the
free gastric mucous membrane.

7. Examine sections of a small intestine in which the
blood-vessels have been injected, and note the capil-
laries running up between the glands of Lieberkühn,
and forming a network in the villi close beneath the
surface.

BILE.

Constituents and Reaction.

Take fresh ox-gall and dilute it with four times its bulk
of water.

1. Test the reaction with litmus paper.   If fresh it is
    distinctly *alkaline.*

2. To a small quantity add acetic acid.   A curdy precipi-
    tate of *mucin* coloured with the bile-pigment will be
    thrown down.   Since the mucin of bile is not ob-
    tained from the liver but from the mucous glands and
    cells in the gall-bladder and duct, the longer the
    bile has been in the gall-bladder the greater the pre-
    cipitate which will be obtained.

3. *Gmelin's test for bile-pigment.*   To a small quantity
    add gradually, drop by drop, shaking after each drop,
    some nitric acid, yellow with nitrous acid; the yellowish
    green colour gradually becomes first a bluish green,
    then blue, then violet, then a dull red, and finally a
    dirty yellow.   Another method is to pour the acid
    carefully over a small quantity of bile in a test-tube,
    or to let a drop of bile mingle with one of acid on a
    porcelain slab; where the fluids mingle, zones of colour,

green, blue, violet, red and yellow, from the bile to the
acid, are seen.

This reaction is more strikingly shewn with yellow bile,
taken direct from the body of a dog or rabbit and so
free from mucus. The first change then is from yellow
to green.

4. *Pettenkofer's test for bile-salts.* Take a little bile,
or preferably a drop of prepared bile-salts, and place
it in a small porcelain basin with a few drops of water
and a *very little* cane-sugar powdered, or in syrup.
Add strong sulphuric acid drop by drop, continually
stirring and keeping the temperature at about 70°C.
The acid first causes a cloudy precipitate of cholic
acid, and then dissolves it, giving a characteristic
purple colour.

5. *Preparation of bile-salts.* Rub ox-gall with animal
charcoal into a thin paste. Evaporate on a water
bath to complete dryness, and exhaust with absolute
alcohol. The alcoholic filtrate should be colourless.
Add to it anhydrous ether as long as any precipitate
is produced, and let it stand. The precipitate either
crystallizes out or falls to the bottom as a thick
viscid syrup; it is a mixture of sodium glycocholate
and taurocholate.

Properties.

6. Moisten a small filter *A* with water, another *B* with
a watery solution of bile-salts. Pour into each filter
the same quantity of olive-oil. Take care that neither
filter becomes dry, by moistening occasionally the out-
side. In a few hours some of the oil will have passed
through *B*, but none at all through *A*.

7. Shake up equal quantities of ox-gall and oil. An emulsion will be formed, and will remain for some time; but eventually the two liquids will separate. For comparison, shake up equal quantities of water and oil; the two liquids will separate much more quickly.

8. Digest some fibrin in gastric juice, and to the acid mixture when digestion is nearly complete, add bile, or a solution of bile-salts. A copious precipitate of parapeptone takes place. The clear supernatant liquid, even when acidified again, will not be peptic, the pepsin having been carried down with the precipitate.

<div align="center">PANCREATIC JUICE.</div>

Artificial Pancreatic Juice.

1. Mince a piece of any fresh pancreas, and with luke-warm water make an infusion. If the infusion is at all acid, neutralize with sodium carbonate. Strain it through muslin. It will have all the properties of pancreatic juice, but its power of acting on proteids (*i. e.* the amount of the proteolytic ferment) will vary greatly with the nutritive state of the pancreas.

2. Make a glycerine extract of pancreas, in the same way as the glycerine extract of gastric mucous membrane was made. It will contain a considerable amount of the amylolytic ferment, and a variable amount (as in § 1) of the proteolytic ferment.

3. Mince finely a piece of fresh pancreas, and rub it up in a mortar with powdered glass or pure sand. For each gramme of gland-substance add 1 cc. of acetic acid 1 p. c., and mix thoroughly in the mortar

for ten minutes. Add ten times the volume of gly-
cerine, and let the whole stand for two or three
days. By treating the pancreas with acetic acid as
above, the 'zymogen' or substance yielding the pro-
teolytic ferment has been converted into ferment, so
that there is a much greater proportion of it in this
extract than in that of § 2.

Properties.

4. Melt some lard, and at a temperature not above 40° C.
   mix one part of melted lard with two parts of the
   pancreatic infusion (§ 1), rubbing them gently toge-
   ther in a warm water bath. A thick creamy emul-
   sion results, which will remain an emulsion even in
   the cold for some hours.

   The glycerine extracts will not cause an emulsion with
   oil, since they do not contain the alkali albuminate
   which is the chief agent in this action.

5. With the glycerine extract (§ 2) make observations
   similar to those made with the amylolytic ferment in
   saliva (Lesson XIV. §§ 3—6).

6. Take two test-tubes, and add to each a few drops of
   the glycerine extract (§ 3) diluted with a 1 p. c. solution
   of sodium carbonate. Boil one (A), leave the other (B)
   unboiled. Place in each a fragment of boiled fibrin,
   and expose to a temperature of 35°. In a short time
   the fibrin in B will have become dissolved, while the
   fibrin in A remains much as it was. In B, peptones
   will be found. The solution of the fibrin by the
   action of the proteolytic ferment of the pancreas
   differs from pepsin digestion in that no transparency
   is produced, and the fibrin appears as if corroded
   rather than dissolved.

DEMONSTRATIONS.

The appearance of the chyle in the lacteals of the mesentery of a rabbit a few hours after a meal,

a. Above the entrance of the pancreatic juice into the intestine,

b. Below the entrance of the pancreatic juice into the intestine.

The thoracic duct.

LESSON XVI.

THE LYMPHATIC SYSTEM.

LYMPHATIC GLANDS.

1. PREPARE vertical sections of a rabbit's **Peyer's patch** which has been hardened in alcohol, and stain them with carmine. When stained, mount a section at once, to observe the immense number of leucocytes; shake up the others in a test-tube with distilled water. Look at them under a low power to see if the leucocytes are for the most part shaken out; if so, mount them in glycerine. Select a comparatively isolated follicle, and observe

    a. The adenoid tissue of the follicle, continuous more or less distinctly with the neighbouring adenoid tissue, and resembling it in all respects except that the fibres are, as a rule, finer, and the meshes smaller.

    b. Round parts of the follicle, narrow spaces between it and the surrounding tissue; these represent the **lymph-sinus** on the outside of the follicle.

    c. The leucocytes scattered on the outside of, but especially abundant within, the follicle.

2. Examine prepared sections of a Peyer's patch with the blood-vessels injected. Note the small arteries passing into each follicle, and dividing into a number of capillaries which run nearly to the centre and then curve back again.

3. Examine prepared sections of a Peyer's patch with the lymphatic system injected. Note that the injected material envelopes to a greater or less extent the separate follicles occupying the lymph-sinus spoken of above, and does not penetrate into the interior.

4. Examine sections, stained and unstained, of an ox's mesenteric **lymphatic gland,** hardened in ammonium bichromate or in alcohol alone. Pencil the sections, *i.e.* gently rub them with a fine camel's-hair brush, or gently shake them in a test-tube with distilled water. Mount in glycerine. Observe with a low objective the following general features:

   a. The connective tissue surrounding the gland, and sending in

   b. The **trabeculæ,** which divide the whole gland into a meshwork of **alveoli.**

   c. Towards the exterior the alveoli are large, elongate, and arranged in a radiate manner, forming the **cortex.**

   d. In the centre and round the hilus the alveoli are smaller, and the meshwork close set, forming the **medulla.**

5. Examine sections as thin as possible of a small portion of medulla or cortex. Observe with a high objective.

a.   The trabeculæ, consisting of branching bands
of connective tissue, containing (in the ox) an
abundance of plain muscular fibres.

b.   On either side of each trabecula, and continuous
with it, adenoid tissue, with coarse meshes, and
nuclei at the nodal points.   In the pencilled sec-
tions these coarser meshes contain but few leuco-
cytes; but in those which have not been so
treated they are crowded so that the meshwork
cannot be made out.   This coarse reticulum forms
the **lymph-sinus.**

c.   In the centre of each alveolus, separated from the
trabeculæ by the lymph-sinus, a much finer reti-
culum, the meshes of which are still largely filled
with leucocytes.   This is the so-called **glandular
substance** or **medullary cord.**

6.   Examine sections of glands with

a.   The blood-vessels injected.   The capillaries are
almost entirely confined to the glandular sub-
stance.

b.   The lymphatic vessels injected.   The injected
material fills the lymph-sinuses, and spreads to a
variable extent into the glandular substance.

SPLEEN.

1.   Prepare sections of mammalian spleen which has been
hardened in ammonium bichromate, and examine
them with objective no. 3 or 4.   Observe

a.   Externally, the broad fibrous-looking sheath or
capsule sending in

b.   Large and conspicuous **trabeculæ**, which divide
     the spleen into **alveoli**; these are much more
     irregular than those of the lymphatic glands.

c.   The **splenic pulp,** filling the alveoli; it resembles
     the glandular substance of the lymphatic gland,
     but has a yellowish mottled appearance; in it
     will be seen round colourless masses of tissue, the
     Malpighian bodies.

2.   Pencil a thin section and examine it with objective
     no. 7 or 8.   Observe

          The splenic pulp consisting of a fine connective-
          tissue reticulum, crowded not only with leu-
          cocytes but also with red corpuscles.   It is the
          presence of red corpuscles which gives the spleen
          the mottled appearance observed under a low
          power.   Note that the pencilling has not brought
          into view any distinct lymph-paths in the alveoli,
          as it did in those of the lymphatic glands.

3.   Cut across a fresh spleen and examine it with the
     naked eye.   Note the white **Malpighian corpuscles.**
     Cut out one with curved scissors, and examine it in
     normal salt solution.   It will be seen to consist of
     adenoid tissue crowded with leucocytes, and to be a
     modification and development of the tunica adventitia
     of a small artery.

4.   Tease out a fragment of fresh spleen.   Note that the
     trabeculæ consist of white fibrous tissue, a few elastic
     fibres and a varying amount of unstriped muscular
     tissue.   In the spleen of some animals, *e.g.* the ox,
     the muscular tissue forms the chief constituent; add
     acetic acid to bring into view their nuclei.

8—2

LYMPHATIC CAPILLARIES.

The lymphatic trunks need not be noticed, since their structure is essentially the same as that of the veins. (Lesson X.)

1.   Lay open the abdomen of a frog, and carefully remove the intestines, and if necessary the oviducts. It will be seen that the peritoneum above and to the outside of the kidney is not adherent to the lumbar muscles, but is separated by a space—the cisterna magna lymphatica. Cut out the piece of peritoneum which forms the partition between this space and the general peritoneal cavity, pin it carefully out flat over a ring of cork or paraffin, and immerse it for ten minutes in silver nitrate solution, ·5 p.c. Wash it well with distilled water, and expose to light. After twenty-four hours, divide it in two pieces, mount them in glycerine, one (*a*) with the peritoneal surface uppermost, the other (*b*) with the cisterna surface uppermost. Observe

in (*a*)

α.   The peritoneal epithelium, consisting of large flat cells with a slightly sinuous outline. This is the usual character of the so-called endothelial cells.

β.   Where several more or less triangular cells seem to radiate from a common point, note at their apices the small nucleated cells surrounding the orifice or **stoma** leading from the peritoneum to the cisterna.

in (*b*)

   α.   The lymphatic epithelium, consisting of flat
         cells, smaller than the peritoneal epithelium,
         and with a very irregular sinuous outline.

   β.   The stomata; very much as in (*a*).

2.   Look at prepared specimens of the diaphragm of a
     rabbit or guinea-pig stained with nitrate of silver
     (Hdb. p. 112), the peritoneal surface being upper-
     most.   Observe

   a.   The bundles of fibres forming the tendon of the
          diaphragm.   The spaces between these mark for
          the most part the course of the lymphatic vessels
          and capillaries.

   b.   The peritoneal epithelium, consisting of flat poly-
          gonal cells.   These are larger over the tendinous
          bundles than over the intervening spaces.   Sto-
          mata similar to those of § 1 *a. β.* may be seen;
          they are chiefly found in the epithelium over the
          intertendinous spaces. Pseudo-stomata, irregular
          patches of staining at the junction of several
          cells, are frequent.

3.   Look at prepared specimens of the diaphragm of a
     rabbit or guinea-pig, pencilled and stained with
     nitrate of silver (Hdb. p. 110), the pleural surface
     being uppermost.   Observe

   a.   The small lymphatic vessels, running between
          the tendinous bundles and lined with somewhat
          irregular spindle-shaped epithelium.   In places
          the curved outline of a valve may be seen.

   b.   The lymphatic capillaries with their character-
          istic sinuous epithelium.

c.   The origin of the lymphatics.   This is best seen
     in specimens deeply stained with nitrate of
     silver, so as to produce the so-called negative
     image.   Note the clear branched spaces, whose
     sinuous outline resembles that of an epithelium-
     cell of a lymphatic capillary.   The clear spaces,
     cavities containing unstained connective-tissue
     corpuscles, stand out in strong contrast with the
     surrounding stained matrix.   The junction of
     these spaces with the interior of a lymphatic
     capillary, may here and there be seen.

LESSON XVII.

LIVER AND GLYCOGEN.

STRUCTURE OF LIVER.

1. PREPARE sections of a liver hardened in Müller's Fluid or potassium bichromate. Observe with a low objective

   a. The division into **lobules.**

   b. The **hepatic cells** and their radiate arrangement in the lobule.

   c. The **intralobular** or **hepatic veinlet.** This frequently appears in the section as a clear space in the centre of the lobule.

   d. The **interlobular** or **portal veinlet**: branches of this, as well as of the **hepatic artery,** are seen between the lobules sending off capillaries towards the hepatic veinlet.

   e. The **bile-ducts,** recognisable by their distinct lining of columnar epithelium.

   The distinctness with which c and d are seen will depend on the plane in which the section passes through the lobules.

2. Examine sections of a liver, the blood-vessels of which have been injected both from the hepatic vein (red

or blue) and from the portal vein (blue or red).
Observe, comparing with uninjected specimens,

a.   The hepatic veinlet, seen according to the plane
     in which the lobule is cut, either as a more or
     less circular section, or as a short veinlet passing
     from the centre of the lobule to the sublobular
     vein.

b.   The portal veinlet running on the outside of the
     lobule.

c.   The radial capillary network uniting the portal
     and hepatic veinlets.

3.   Prepare very thin sections of the uninjected liver, and
     examine with a high objective.   Observe

a.   The polygonal outline of the hepatic cells.

b.   Their granular protoplasm, generally containing
     oil-drops.

c.   Their spheroidal nuclei.   Many cells contain two.

d.   The capillary blood-vessels, traceable by their
     containing blood-corpuscles, running out from
     the centre of the lobule between the rows of
     cells, and joining with one another at intervals
     by cross branches.

e.   The bile-ducts, their columnar epithelium with
     distinct nuclei.   In some of the sections the epi-
     thelium may be seen to become shorter and more
     cubical as the duct approaches a lobule.   The
     duct itself often appears to end abruptly at the
     margin of the lobule.

4.   Examine sections of a liver injected from the bile-duct.
     The bile-capillaries will be seen within the lobules

as a fine network of minute threads of injected material, passing between and over the surfaces of the cells.

5.   Scrape a small portion of perfectly fresh liver, and examine the hepatic cells, comparing with § 3.

GLYCOGEN.

1.   Open the abdominal cavity of a rabbit *immediately* after death, and, disregarding the bleeding, cut out the liver as rapidly as possible.   Divide one half into pieces of about 2 cc., and throw them at once into a relatively large quantity of boiling water (prepared beforehand). Place the other half on one side in a warm place.   In two or three minutes, when the pieces in the boiling water are all thoroughly coagulated, and the ferment has been destroyed, remove them and pound them in a mortar into a paste ; mix this paste with 500 cc. of boiling water, acidulate with dilute acetic acid, and boil for three minutes. Let it stand till the sediment of proteids has settled, and filter the milky fluid through a coarse filter. The sediment may be squeezed in linen, and the expressed fluid thrown on the filter. This opalescent filtrate is a crude infusion of glycogen. The above will afford material for several students.

If it contains *much* proteid material, it should be carefully neutralized, boiled again, and filtered.

2.   *a.*   Place a few cc. of the fluid in a test-tube, and add a drop or two of a dilute iodine solution.   A port-wine colour will result.   Warm gently ; the colour will disappear, but will return on cooling (unless much proteid matter be present).

  b. Test 5 cc. for sugar with Fehling's fluid : a small quantity only will be found.

  c. Add to 10 cc. a drop of saliva, or artificial pancreatic juice, and place it in the warm chamber; the opalescence will disappear, and the fluid become transparent.

    a. To 5 cc. of this add iodine as before; the port-wine colour will not appear, shewing that glycogen is no longer present.

    β. Test the other 5 cc. for sugar; much more than before will be found.

 3. After some hours' exposure, make a decoction of the other half of the liver. It will probably be *acid*, and so require for neutralization no further addition of acid, but some sodium carbonate.

  a. The decoction will be clear and transparent, not milky.

  b. It will give no port-wine colour with iodine.

  c. It will contain sugar in abundance.

 By a *post-mortem* change, the glycogen which was in the liver at the moment of death, has all become converted into glucose.

DEMONSTRATION.

Artificial Diabetes.

LESSON XVIII.

THE STRUCTURE OF THE LUNG. THE MECHANICS OF RESPIRATION.

1. CUT out a piece of the lung of a freshly-killed newt, and stain it with gold chloride. Mount with the inner surface upwards, and note

   a. The layer of flat epithelial cells,

   b. The underlying connective tissue, with a close plexus of blood-vessels.

2. Distend the lungs of a frog with chromic acid ·5 p. c., tie the trachea, and place the distended lungs in a relatively large quantity of the acid for a week and then treat with alcohol as usual. Stain a piece with hæmatoxylin; when it is well stained, soak it for a short time in bergamot oil, and imbed it in a mixture of spermaceti (1 part) and castor oil (4 parts), and let it cool. Cut sections, using olive oil instead of alcohol to wet the razor. Transfer the sections to a mixture of carbolic acid and turpentine, warm gently, and mount in Canada balsam. Observe with a low objective, the primary, secondary, and tertiary septa forming the alveoli, and with a high power the structure of the wall similar to that of the newt.

3. Make transverse vertical sections from the posterior part of a small mammalian trachea, which has been

hardened in chromic acid ·5 p.c.   The section should
be so taken as to cut through the ends of a tracheal
ring and include the membranous part between them.
Observe

a.   Externally, the **fibrous coat** of loose connective
tissue, becoming denser towards the tube of the
trachea; in this are imbedded the two ends of
the ring of **cartilage.**

b.   Stretching between the ends of the cartilage, a
transverse band of unstriped **muscular fibres;**
there may also be visible outside this the cut
ends of muscular fibres running longitudinally.

c.   The **submucous coat** continuous with the fibrous
coat, but of finer fibres.

d.   The **mucous glands,** the alveoli of which form
almost a continuous layer in the submucous
tissue; the ducts will be less evident, but they
may be seen running towards the surface, and
occasionally opening on it.

e.   The cut ends of **elastic fibres,** which form a
distinct longitudinal layer, on the inner side of
the submucous tissue.   It will be seen that this
is most marked in the membranous part of the
trachea; where, too, the fibres are frequently col-
lected into bundles.

f.   The **mucous coat.**   In this may be seen

a.   A number of fine elastic fibres with a variable
quantity of adenoid tissue, which becoming
more dense towards the surface, forms a dis-
tinct basement membrane for

β. The epithelium, consisting of two, three, or more layers of small cells lying beneath and between the central processes of a layer of columnar ciliated cells. If the trachea has not been hardened and prepared carefully, the cilia may appear only as a granular border lying on the columnar cells. Goblet-cells will probably be seen amongst the ciliated cells.

4. Make longitudinal vertical sections of trachea, taken through the tracheal rings, and compare them with the transverse sections, noting especially the elastic and muscular elements.

5. Make sections, stained and unstained, of prepared mammalian lung, preferably the lung of a fœtus injected with and hardened in chromic acid or potassium bichromate. Observe

a. Sections of blood-vessels (cp. Lesson X.).

b. Sections of bronchia. Note that

The sections of the larger bronchia resemble sections of trachea, except that

α. The cartilages are irregular in form, and irregularly scattered throughout the fibrous coat.

β. The transverse muscular fibres now form a complete ring in the submucous coat.

From the smaller bronchia the cartilages are absent, but the muscular fibres still continue as scattered fibres arranged transversely.

c. The passage of a small bronchial tube into an infundibulum. Note that

α. The columnar ciliated cells become shorter and lose their cilia.

β. The muscular fibres apparently cease.

γ. The longitudinal fibres spread out into an irregular meshwork.

This may be occasionally seen in a section.

d. Sections of alveoli.

α. The outline of the alveoli : the distinct basement membrane.

β. The flat, tesselated epithelium. This will be seen best where a portion of the wall of the alveolus is left flat in the section ; sometimes it may be seen in profile.

γ. The elastic fibres of the alveolar wall.

δ. The nuclei and collapsed walls of the capillaries.

6. Make sections of mammalian lung, injected with and hardened in alcohol acidulated with acetic acid. Observe more closely the abundant elastic fibres of the alveolar walls and the nuclei of the epithelium.

7. Make sections of a lung, the blood-vessels of which have been injected. Observe the close-set capillary network over the alveolus, and the artery forming a circle round the mouth of each alveolus.

DEMONSTRATIONS.

1.   The circulation in the lung of toad.

2.   The movements of the column of air in respiration.

3.   The movements of the diaphragm.

4.   Apnœa.

5.   Slight dyspnœa.

6.   The action of the respiratory muscles in dyspnœa.

7.   The collapse of the lungs on puncturing the thorax, and the consequent futility of the respiratory movements.

8.   The action of the phrenic nerve.

LESSON XIX.

THE COLOUR OF BLOOD. RESPIRATION.

1. PLACE a rat or guinea-pig under a bell-jar with a sponge moistened with chloroform. When it is thoroughly under the influence of the chloroform, quickly open the thorax, and cut across the heart. Collect the blood in a glass beaker, and whip it; pour one half of the defibrinated blood into a platinum crucible surrounded by a mixture of ice and salt. Leave it till it is frozen. Then remove it to a not too warm place, so that it may thaw slowly. By this means the blood-corpuscles will be broken up, and the blood will become of a dark 'laky' colour. If the blood does not become thoroughly laky it should be frozen and thawed again.

   Compare the dark colour and transparency of this laky blood with the scarlet colour and opacity of the remaining half of the animal's blood which has been simply defibrinated. The opacity or transparency may be easily judged by attempting to read type through a drop placed on a glass slide.

2. Place the laky blood on one side in a cool place (a temperature of 0° C. is preferable) for 24 hours. A sediment will then have formed consisting partly of (oxyhæmoglobin) **hæmoglobin crystals** and partly of

broken up corpuscles. Or a drop of the blood, allowed
to evaporate on a slide, will be found to contain
crystals.

3. Examine the sediment under the microscope for oxy-
    hæmoglobin crystals. Note

    a. Their form. In the rat they are thin rhombic
        prisms, often almost needle-shaped.
        In the guinea-pig they have the appearance of
        tetrahedra, but in reality belong to the rhombic
        system.

    b. Their colour. It is that of arterial blood.

4. Examine the sediment for the stroma of the red
    blood-corpuscles. These will be seen as pale colour-
    less rings mixed up with a good deal of granular
    débris.

5. Prepare a solution of oxyhæmoglobin. This may be
    done by dissolving the crystals in distilled water. In
    the absence of crystals the following method may be
    adopted.

    Take a fresh blood-clot, and drain off as much serum
    as possible. Cut it into small pieces. Wash it with
    water, and throw away the first two washings. Treat
    it then with water, squeezing the pieces, and filter
    through a coarse filter.

6. Examine, by means of a spectroscope[1], the **absorption
    bands** of oxyhæmoglobin in a very dilute solution.
    Previously determine the position on the scale of the
    lines B, C, D, E, F, by examining the solar spectrum.
    If this is not practicable, determine at least the posi-

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[1] The demonstrator will show the method of using the instrument.

L.   9

tion of D, by burning a sodium flame in front of the collimator, and bringing the micrometer wire to coincide with the sodium line.

  a. Note the two absorption bands; one (a) towards the red, the narrower but the denser of the two, and the first to appear; the other (β) towards the blue, broader, but less dense.

  b. Note the position of the bands, both to the blue side of D, lying between D and E.

7. Increase gradually the strength of the solution.

  a. The spectrum is more and more cut off both at the blue and at the red end, but especially at the former. The absorption bands are both blacker and broader.

  b. As the solution becomes stronger, the two bands run together, while the two ends also suffering absorption, light passes through only in the green to the blue side of E or b, and in the red, to the red side of D.

  c. With a still stronger solution, the green light also is absorbed, and only the red is visible, and this at last disappears.

8. Reduce the oxyhæmoglobin solution with Stokes's iron[1] fluid in the cold, or with a few drops of ammonium sulphide solution in the warm chamber.

  a. Compare the claret colour of the **reduced** hæmoglobin solution with the bright scarlet of the original solution.

  b. Examine with the spectroscope. There is a single broad band, occupying a position inter-

[1] See Appendix.

mediate between those of the two oxyhæmoglobin bands which have disappeared. The band is not quite intermediate; its mid-line lies nearer *D* than *E*. This single band is much less dark than either of the two bands produced by the same quantity of oxyhæmoglobin.

c. With stronger solutions, less of the blue end disappears. Instead of the green beyond *E* or *b* remaining, it is the blue beyond *F* which now remains.

9. Pour the reduced solution two or three times from one vessel into another so as to expose it well to air. The bright scarlet colour will be restored; the oxyhæmoglobin spectrum will reappear.

If allowed to remain at rest, reduction (from excess of reducing reagent present) soon returns.

10. Examine the spectrum of blood-crystals either with the microspectroscope or by placing a thick layer of crystals on a glass slide before the larger spectroscope. The spectrum of oxyhæmoglobin is seen.

11. Pass carbonic oxide through a oxyhæmoglobin solution for some little time.

a. Note the peculiar bluish tinge acquired. Examine the spectrum; two bands are seen like those of oxyhæmoglobin, but both placed more towards the blue end.

To compare their position, place some of the oxyhæmoglobin solution before the spectroscope, bring the micrometer wire *to the middle* of one of the bands, and fix the telescope in position. Replace the oxyhæmoglobin solution by the car-

9—2

bonic oxide hæmoglobin solution and examine; the middle of the band will now be to the blue side of the wire.

b. Treat the carbonic oxide hæmoglobin with either of the reducing agents used above. Reduction will not take place.

DEMONSTRATIONS.

The spectra of

    a. Oxy-hæmatin.

    b. Reduced hæmatin.

The gases of the blood.

The colour of venous and arterial blood in the living animal, and its dependence on the presence of oxygen in the lungs.

The respiratory function of the pneumogastric.

The action of the respiratory centre.

The effect of arterial and venous blood on the tissues.

The phenomena of asphyxia.

LESSON XX.

STRUCTURE OF THE KIDNEY.

1. FROM a kidney hardened in ammonium bichromate, prepare radial sections extending from the pelvis to the external surface. If any difficulty is experienced in cutting the sections, a microtome may be used. Observe with a low power

    a.   The **medulla.** Note the straight tubes, the **tubuli uriniferi recti,** radiating from the pelvis outwards. These, increasing in number by division, form the **pyramids of Malpighi.**

    b.   The **cortex.** Note

        a.   The pyramids of Malpighi, dividing at their base into a number of bundles, the **pyramids of Ferrein.** These, radiating outwards and becoming thinner, are lost as they approach the surface of the kidney.

        β.   The convoluted tubes, the **tubuli uriniferi contorti,** occupying the intervals between the pyramids of Ferrein, and forming a layer at the external surface.

        γ.   The **Malpighian capsules,** with their **glomeruli,** dispersed in double rows in the intervals between the pyramids of Ferrein.

The part of the cortex in which are the pyramids of Ferrein is frequently spoken of as the **marginal zone.** The part of the cortex containing the convoluted tubules is often distinguished from the pyramids of Ferrein by the name of **cortical substance.**

2.   Prepare very thin radial sections of the medulla and of the cortex. Stain some with hæmatoxylin, and leave others unstained.

A.   In the cortical substance observe

  a.   The **Malpighian capsules.** Note in each

   a.   The external wall of the capsule and the nuclei of the lining epithelium.

   β.   The glomerulus; the outline and nuclei of the blood-vessels (the nuclei of the investing epithelium).

   γ.   The narrow neck of the capsule. This is seen only when the section passes through the capsule in a particular plane.

  b.   The tubuli contorti: **secreting tubules.** Note in these that

   a.   The limits of the individual cells lining the tubules are very slightly shewn; a distinct cell-membrane being absent.

   β.   The cells consist of granular protoplasm, the outer part of which is often distinctly and coarsely striated.

   γ.   The lumen of the tubule is frequently indistinguishable. The nuclei are round, and more or less obscured by the striation.

B. In the medulla,

    c.   The straight tubules : **collecting** or **discharging tubules.**   Note in these

        α.   The short columnar or cubical epithelium, with round nuclei and well-defined outlines, leaving a considerable lumen in the centre of the tubule.

        β.   The distinct basement membrane.

C. In the pyramids of Ferrein,

    d.   The **looped tubes of Henle,** consisting of

        α.   The **descending limb.** This may be recognized by its narrow calibre, and its transparent flattened epithelium, the nuclei of which project on alternate sides into the lumen, somewhat after the manner of a capillary blood-vessel. The basement membrane of the tubule is thick and conspicuous.

        β.   The **ascending limb.** This is wider than the descending limb, and in many cases does not differ in appearance from a collecting tube ; frequently, however, its epithelium is imbricate. The nuclei of the cells are generally oval.

        The change of character of the epithelium of the loop of Henle may take place either in the descending or in the ascending limb.

    e.   The **connecting tubules.** These differ little from the secreting tubules, save that their course is zigzag, their bends being angular and not rounded.

Try and make out the following course of a
tubule. The capsule is continuous by a narrow
neck with a secreting tubule. This, after wind-
ing about in the cortical substance, descends
(along a pyramid of Ferrein) towards the me-
dulla as a descending limb of a Henle's loop,
returns as an ascending limb, and, after a short
course as a connecting tubule, redescends as a
collecting tubule, and finally, by junction with
others, becomes a discharging tube. No tube can
be thus followed in a section, but the various
transitions may be seen.

3. In sections through the cortex, and in tangential
sections through the medulla, observe

   a. The firm, conspicuous, transparent membrana
propria of the tubuli ; often seen devoid of epi-
thelium. This is thick in the loops of Henle,
and thin in the other parts of the tubules.

   b. The nuclei of the membrana propria of the
tubules, and of the interstitial connective tissue
or stroma.

   c. The characters of the epithelium in the different
tubules. Try to distinguish, in the sections of
the pyramids of Ferrein, between the loops of
Henle and the collecting tubules.

   d. The transverse, and other sections of the blood-
vessels.

4. Examine sections of kidney injected from the renal
artery. Observe

    a.   The branches of the renal artery running between the Malpighian pyramids.

    b.   The **interlobular** branches, continuing outwards in the intervals between the pyramids of Ferrein, and giving off on either side small **afferent** arteries to the Malpighian bodies.

    c.   The single **efferent** vessel issuing from each glomerulus, and almost immediately dividing into capillaries, which form a close net-work round the tubules.

5.   Cut as thin a section as possible of a fresh kidney, and tease it out in normal saline solution.   Observe

    a.   The characters of the cells.

    b.   The basement membrane of the tubules.

6.   Cut vertical sections of a rabbit's or dog's **bladder,** hardened in ammonium bichromate, and observe

    a.   Externally the peritoneum.

    b.   The muscular coat, consisting of two layers, the outer more or less distinctly longitudinal, the inner more or less distinctly transverse.

    c.   The submucous coat.

    d.   The mucous coat. Note that the epithelium consists of three or more rows of cells.

        α.   An outer, generally cubical, layer.

        β.   A layer of pear-shaped cells.

        γ.   Small elongated cells between the central processes of the pear-shaped cells.

7. Scrape the internal surface of the mucous membrane,
    and observe more particularly the character of the
    epithelial cells.

8. Cut transverse vertical sections of a rabbit's or dog's
    ureter. Note that the structure is much the same as
    that of the bladder, but the longitudinal muscular
    coat is generally indistinct, and the submucous coat
    very loose.

LESSON XXI.

URINE.

1.   TEST the reaction of fresh urine with litmus paper, it will be **acid**; this is mainly due to the presence of acid sodium phosphate, and *not* to free acid.

2.   Put in a warm place 200 cc. of urine, and observe from time to time.

   a.   It will, after twenty-four or more hours, lose its acid reaction, and become alkaline. Gently warm the litmus paper turned blue by the urine, the blue colour will disappear, shewing that the alkalinity is due to the presence of ammonia or a salt of ammonium.

   b.   It will gradually become cloudy, and yield a deposit of various salts.

   c.   Its odour will become putrefactive.

   The urine has undergone **alkaline fermentation.**

3.   A small quantity of **mucus** derived from the urinary passages is occasionally present in the form of a faint cloudy precipitate. This may be rendered more opaque by the addition of acetic acid.

4. **Urea.** Place a few crystals of urea in a watch-glass, and dissolve them in a small quantity of water.

a. Let a drop of the solution evaporate on a glass slide, and observe under the microscope the crystals of urea, consisting of four-sided prisms. (Hdb. fig. 322.)

b. Add to another drop on a slide a drop of pure strong nitric acid; comparatively insoluble six-sided tablets of nitrate of urea will crystallise out. (Hdb. fig. 323.) Note the striation frequently present in these tablets.

c. Repeat (*b*), using oxalic instead of nitric acid, Rhombic tablets of oxalate of urea will be formed. (Hdb. fig. 324.)

d. Dilute considerably the remaining solution, and add to it a solution of mercuric nitrate. A white precipitate of mercuric oxide combined with urea will take place.

5. Evaporate 20 cc. of urine in a water-bath till its volume is reduced to about 2 cc.   Filter, and examine the filtrate for urea (§ 4, *b. c.*)

6. **Uric Acid.**

a. Place a few crystals of prepared uric acid on a slide, and add a little liquor potassæ to dissolve them ; then add nitric acid just to excess. Crystals of uric acid will be deposited, which may be examined under the microscope. They are generally rhombic plates with obtuse angles, but they vary largely (Hdb. fig. 305). Note the star-shaped and dumb-bell crystals.

    b. Dissolve some uric acid in an evaporating dish, in a little dilute nitric acid, and evaporate nearly to dryness at a low heat in a water-bath. Add a drop or two of *weak* ammonia; a red purple colour will be formed. This is called the Murexide test for uric acid. If a drop of caustic potash be added the colour will turn to a blue purple.

7. Evaporate 50 cc. of urine to one-third its bulk; filter, and add to the filtrate a few drops of pure strong hydrochloric acid; set it aside till a red deposit is observed. Examine the deposit under the microscope for uric acid crystals, and subsequently apply the tests § 6, *a. b.*

TESTS FOR SOME ABNORMAL CONSTITUENTS OF THE URINE.

8. **Albumin.** The albuminous bodies found in the urine have, with rare exceptions, the reaction of serum albumin. Hence apply the tests for that substance. Lesson III. § 10.

9. **Sugar.** Diabetic urine. Coagulate any albumin that may be present, by boiling. Filter through a little animal charcoal, and test with Fehling's fluid.

10. **Bile Pigment.** Use Gmelin's test. (Lesson XV.)

11. **Bile-acids.** Soak a small piece of white blotting paper in a syrup of sugar, and allow it to dry. Let fall on it a drop of the previously concentrated urine supposed to contain bile-acids, and near it a drop of strong sulphuric acid, so that the two drops run into one another. If bile-acids are present, there will be

a purple colouration at the junction of the drops. This ready method can be adopted only when the quantity of bile-acids present is large.

QUANTITATIVE ESTIMATION OF UREA (LIEBIG'S METHOD).

12. The following standard solutions will be previously prepared by the Demonstrator.

a. A baryta mixture, consisting of two volumes of barium nitrate, saturated in the cold, and one volume of barium hydrate also saturated in the cold.

b. A solution of mercuric nitrate of such a strength that 1 cc. precipitates 10 mgr. of urea.

Take 40 cc. of urine, and add to it 20 cc. of the baryta mixture. A copious precipitate of barium sulphate, phosphate, etc. will fall. Filter and keep the filtrate. Fill a Mohr's burette with the mercuric nitrate solution, and let the solution fall drop by drop into 15 cc. of the filtrate, stirring constantly.

Have ready a glass plate on a dull black surface with a number of drops of sodium carbonate solution on it; from time to time add a drop of the urine to one of the sodium carbonate. When a yellow colour is thus produced, the mercuric nitrate is in excess, and all the urea has been precipitated. Since each cc. of the mercuric nitrate solution precipitates 10 mgr. of urea, the number used in the experiment multiplied by 10 gives the number of mgr. in 15 cc. of the filtrate, that is in 10 cc. of urine. But there is sodium chloride in the urine, and when this salt is

mixed with mercuric nitrate a double decomposition takes place; thus, until the quantity of sodium chloride present is exhausted, the mercuric nitrate is no longer available for precipitating the urea. Now the amount of sodium chloride in urine is tolerably constant; in each 10 cc. there is enough to act upon 2 cc. of the standard mercury solution. *Hence in reckoning the number of cubic centimetres of standard solution used, a deduction of 2 cc. must always be made.*

The method thus carried out is only approximately correct.

QUANTITATIVE ESTIMATION OF SUGAR.

13. Take the previously prepared standard solution of Fehling's fluid. This is of such a strength that 10 cc. are equivalent to ·05 grm. of grape-sugar, *i.e.* 10 cc. contain just that amount of cupric oxide which ·05 grm. of grape-sugar is capable of reducing to cuprous oxide: the cuprous oxide is precipitated, and the fluid is decolorized.

Take 10 cc. of diabetic urine, from which any albumin which may be present must be removed, and add to it 90 cc. of distilled water; place this in a burette.

Pour into a beaker exactly 10 cc. of the standard solution, dilute it with four or five times its bulk of distilled water, and boil.

Then run into it from the burette 40 cc. of the diluted urine, and boil again; let the precipitate settle, and look through the fluid at a sheet of white paper; if it is still blue, add another cc. from the burette and boil once more, and so on till the supernatant fluid

ceases to have a blue tinge; taking care, towards the end of the process, to add a few drops only, each time. If after adding the 40 cc. and boiling, the fluid is decolorized, too much urine has been added, and another 10 cc. of the standard solution must be measured out, running in, say 20, instead of 40 cc. of diluted urine.

Avoid delay in judging of the presence of the blue colour after each boiling; since on cooling the blue colour may partially return, and hence the amount of sugar present be estimated too highly. It is best to make two or three determinations; the first rapid and rough, the others more exact.

Suppose that the number of cc. of urine has been found which is just sufficient to decolorize the standard solution taken; that volume then contains just sufficient sugar to reduce the cupric oxide contained in 10 cc. of the standard solution to cuprous oxide; and we have seen that it requires ·05 grm. of sugar to do this, therefore the number of cc. used contains ·05 grm. of sugar, but of this one-tenth only was diabetic urine, therefore one-tenth of the number of cc. used gives the number of cc. of urine containing ·05 grm. of sugar; from which the percentage can easily be calculated.

LESSON XXII.

SKIN AND TOUCH.

SKIN.

1. Prepare vertical sections of human scalp which has
    been hardened by placing it in chromic acid ·2 p.c.
    for a week. Stain the sections with picrocarmine,
    mount them in glycerine and observe

A. The **Epidermis**: proceding from the outside will be
    seen

   a. Cells without protoplasm, and apparently with-
         out a nucleus, very much flattened so that they
         present the appearance of a number of fibres.
         This is called the **horny layer;** in the stained
         sections it will be yellow.

   b. Nucleated cells, more or less spheroidal in form.
         Some of these cells are frequently seen to be
         serrated or marked with ridges.

   c. A row of columnar cells placed perpendicularly
         to the tissue beneath, and having no distinct cell-
         membrane. The spheroidal and the columnar
         cells together form the **Malpighian layer.**

L.   10

B.   The **Dermis**: in which may be seen

    a.   A thin layer of imperfectly differentiated connec-
tive tissue, the corpuscles of which are imbedded
in an almost hyaline, very slightly fibrillated
matrix.   The upper portions of this form a kind
of basement or limiting membrane immediately
below the columnar epidermic cells.   The lower
portions pass gradually into

    b.   A fine meshwork of connective tissue, becoming
coarser in the deeper parts, and there continuous
with the subcutaneous connective tissue.   The
dermis is considered to cease with the appearance
of fat-cells in the connective-tissue meshwork;
but there is no definite line of demarcation, and
in some places the deposit of fat does not
occur.

    c.   Numerous elastic fibres, present both in *a* and *b*.

    d.   The **papillæ**: conical elevations, composed of
a and *b*, rising up into the Malpighian layer,
which therefore appears to dip down between
them.

    e.   Indications of the blood-vessels, abundantly dis-
tributed all over the dermis, and forming ca-
pillary loops in the papillæ.   These are better
seen in injected specimens.

    f.   Nerve-fibres, for the most part becoming lost to
view as they approach *a*.   Some may be seen
entering the papillæ.

    g.   **Hair-follicles** and the roots of hairs.   Note

        a.   The external sheath of the follicle formed
of fibrous tissue continuous with that of

the dermis, but much denser in arrangement.

β.   The internal sheath of the follicle, consisting for the most part of indistinct transversely arranged fibres, amongst which oblong nuclei (of plain muscular fibres) may be discerned.

γ.   A hyaline limiting membrane more marked than that of the dermis.

δ.   The external root-sheath, consisting of cells continuous with, and resembling those of the Malpighian layer.

ε.   The internal root-sheath, pale and transparent, passing into the horny layer of the epidermis. At the exit of the hair from the skin the horny layer dips down around it in the form of a cone.

ζ.   The papilla of the hair-follicle, consisting of connective tissue, with numerous cells, proceeding from the internal sheath of the follicle.

h.   The external root-sheath near the top has a diverticulum, which constitutes a **sebaceous gland.** There may be more than one.

In the deeper part of the gland the cells become cubical.

i.   Below the base of the gland may be seen a band of plain muscular fibres, stretching obliquely from the inner sheath of the follicle to the upper part of the dermis.

10—2

2. Cut sections of the palm of the hand or of the fore-
head, hardened in chromic acid, picric acid, or
alcohol, to observe the structure of the **sweat-
glands.** Note

   a. The terminal gland-knot in the subcutaneous
   tissue with its membrana propria continuous with
   that of the dermis, and lined by a single layer
   of cubical cells.

   b. The gland-tube having columnar epithelium
   continuous with the cells of the Malpighian
   layer of the epidermis. In the epidermis the
   tube has no wall except that formed by the
   concentrically arranged epidermic cells ; its
   course here is spiral.

3. Prepare the cornea of a frog as in Lesson v., B. § 4.
Observe

   a. A meshwork of nerves, without medulla, but
   with primitive sheath recognised by its nuclei
   and perineurium. This forms the **primary nerve-
   plexus,** it is at a slightly deeper level than

   b. A meshwork of nerves with much closer meshes,
   consisting of fine axis-cylinders, without a primi-
   tive sheath, but having numerous varicosities in
   their course. This forms the **sub-epithelial
   nerve-plexus.**

4. Cut out the cornea of a recently killed rabbit, taking
care not to drag it, and place it in gold chloride for
an hour. Wash well and expose to light as usual,
but do not pencil it. When it is well stained, if
necessary harden with alcohol, imbed a piece of it
vertically in paraffin, and cut sections as thin as
possible. Observe

The primary nerve-plexus in the connective tissue, giving off branches which divide and form the sub-epithelial nerve-plexus, immediately underneath the epithelium; from this very fine fibrils are given off, which, penetrating between the epithelial cells, form there the **epithelial plexus.**

This may be considered as the type of nerve-termination in those parts of the skin where there are no special terminal organs.

5. Cut sections of the tips of fingers which have been kept in potassium bichromate 1 p. c. for a day, to observe special nerve-endings :

A. **Touch-corpuscles.**   Note that

a. They are oval in form, and are found in the axes of the papillæ; many papillæ are without them, in which case a blood-vessel may generally be seen running up into the papilla.

b. They appear to consist of a transparent matrix in which nuclei are disposed transversely.

c. The medullated nerve-fibre enters near the base, and may be traced coiling round it spirally for a variable distance.

To observe the course of the nerve-fibre satisfactorily, a small piece of fresh skin from the tip of a finger should be placed in osmic acid 1 p. c. for about twelve hours till it is quite black, and then treated with alcohol as usual. The dark nerve-fibres can be distinctly seen entering the touch-corpuscles; and occasionally in papillæ without these bodies, one or more nerve-fibres may be seen running up the papilla as far as the Malpighian layer of the epidermis; its

further course amongst the cells will probably not be
made out in these preparations. Osmic acid speci-
mens, after they have been well washed, may be
mounted in glycerine, or in a saturated solution of
acetate of potash or after clearing in Canada balsam.

B.   **Pacinian bodies.**   These occur in the subcutaneous
tissue; but their structure can be more readily studied
in the mesentery of a cat, in which they appear as trans-
parent oval bodies generally surrounded with fat.   Ob-
serve

  a.   The twisting medullated nerve-fibre and the
        thickening of its connective-tissue sheath as it
        approaches the Pacinian body.

  b.   The division of the connective-tissue sheath to
        form the capsules.

  c.   The loss of medulla as the sheath splits up.

  d.   The colloid body inside the inner capsule.

  e.   The axis-cylinder penetrating the colloid body,
        and ending in it in a slight enlargement.

TOUCH.

1.   **Power of localisation.**

Using a pair of small bone or wooden compasses, deter-
mine the distance at which the points must be placed
from each other in order that they may be felt as
two points when applied lightly and with the same
force to various parts of the body.   Try if the follow-
ing order is correct: tip of tongue, tips of fingers, palm
of hand, cheeks, back of hand, fore-head, front of fore-
arm, back of fore-arm, back of neck, &c.

2. **Sensation of temperature.**

Dip a small metallic knob in hot water (e.g. 70° C.), and applying it to the same parts of the body as before, determine roughly the sensitiveness to temperature of these parts.

3. **Estimation of weight.   The muscular sense.**

> *a.*   Determine the smallest difference in weight which can be appreciated when the weight is held in the hand.

> *b.*   Then laying the hand and arm supine on a table, so that they are motionless, and placing weights on the palm of hand, determine as before the difference of weight which can be appreciated. This will be much less in the case of (*b*) than of (*a*). In (*a*) the muscular sense is employed ; in (*b*), only the sensation of pressure. The weight should be placed on the same part of the palm of the hand in each case.

4.   Estimate (in either way) the difference of weight which can be appreciated

> *a.*   When the weights are light, *e. g.* 1, 2, 3, 4, or 5 grms.

> *β.*   When the weights are heavy, *e.g.* 10, 20, 30, &c., or 100, 200, &c. grms.

Smaller differences will be appreciated in *a* than in *β*. In fact the difference of weight which can be appreciated bears, roughly speaking, a constant ratio to the whole weight.

5.   Plunge the finger into mercury, and then draw it out. The sensation will be that of a ring passing down and

up the finger, the greatest sensation being where there is the greatest variation of pressure.

6. Place two equal light weights, one cold the other warm, on the corresponding fingers of the hands; the cold one will feel the heavier.

7. Paint a ring of collodion on the palm of the hand, leaving only a small central space, and attempt to distinguish between the approach of a hot body and gentle contact of a feather.

8. Place the elbow first in warm water, and then in a mixture of ice and water. There will be a sensation of pain in the fingers and cold in the elbow. The application of cold to the *trunk* of a nerve does not cause a sensation of cold.

9. **Tactile delusions.**

Cross the second finger over the first or the third over the second, and place between their tips a small marble (or any solid round body a little larger than a pea) so that it touches the radial side of the first, and the ulnar side of the second finger-tip. On gently rolling the body about, a sensation as if of two distinct bodies will be felt.

The same delusion is experienced if the tip of the nose be gently rubbed with the tips of the fingers so placed.

Experiments 6 and 7 will need the assistance of a second person.

LESSON XXIII.

OLFACTORY AND GUSTATORY ORGANS.

1. Lay bare the olfactory chambers of a newt, and place
   them in osmic acid 1 p.c. for 24 hours. Tease out
   in water a small piece of the mucous membrane.
   Or place in Müller's fluid for a week or more, and
   tease out in the same fluid or in water. Observe

   a. The **cylindrical** epithelium **cell**, its large oval
   nucleus, its broad, clear, peripheral process, and
   its branching, faintly granular, central process.

   b. The **rod-cell**, with its spherical nucleus, its hya-
   line slender peripheral, and its still more slender
   varicose and branching central process.

   c. Groups of the nuclei of *b*, forming the nuclear
   layer (2 B. *b*) lying in the meshes of a reticulate
   slightly granular protoplasm.

   d. Cylindrical cells will frequently be seen, sur-
   rounded and partially hidden by numerous rod-
   cells.

2. Remove the lower jaw from the head of a newt,
   cut off the nose by an incision carried just in front of
   the eyes. Place it in chromic acid ·5 p. c. for three
   days. Imbed it vertically, and cut transverse sections,

until the olfactory chambers are reached. These may be at once recognised by their peculiar epithelium. Observe that

A   In the non-olfactory portion of the mucous membrane

    a.   The surface-epithelium is cylindrical and ciliated, with the exception of a certain number of goblet-cells.

    b.   Below the ciliated cells there is a single row of small spherical granular nucleated cells.

B.   In the olfactory part of the mucous membrane the division of the epithelium into an outer and an inner layer.

    a.   The outer layer consisting of the peripheral processes of both cylindrical and rod-cells.

    b.   The inner or nuclear layer consisting of several rows of nuclei belonging to the rod-cells. The nuclei of the cylindrical cells form a single row in the upper part of this layer, but these, as well as the processes of the lower rod-cells, are generally hidden by the nuclei of the rod-cells. In parts of the section where the nuclei have fallen away the branched central processes of the cylindrical cells may be seen to form an irregular meshwork.

    c.   Beneath the epithelium will be seen fine connective tissue, in which lie cut bundles of plain non-medullated nerve-fibres.

A great number of simple mucous glands will be seen beneath the mucous membrane both of A and B.

3.   From a rabbit's tongue which has been hardened in
     chromic acid or potassium bichromate excise a papilla
     foliata and imbed in such a way that the sections
     will be at right angles to the ridges. The papilla
     will easily be recognized as an oval patch strongly
     marked with ridges, about 3 or 4 mm. in its longest
     diameter, at the side of the back of the tongue.

     Prepare thin vertical sections and observe

a.   The section of the ridges with the intervening
       fossæ.

b.   In each ridge three papilliform processes of the
       dermis, one median, two lateral.

c.   The horny epidermis, consisting of cells very
       flattened, but still retaining their nuclei, cover-
       ing the ridges and lining the fossæ.

d.   The Malpighian layer of the epidermis under-
       neath the horny layer in the above situations,
       and also filling up the hollows between the
       lateral and median papillæ of the ridges. (Cf. *c*
       and *d* with Skin Lesson XXII.).

e.   On the outside of each lateral papilla, and
       therefore lining each side of the fossa, the row
       of **taste-bulbs.**

f.   Each bulb reaching from the dermis of the papilla
       to the free surface of the horny layer lining the
       fossa, where its circular open mouth may be
       often seen.

g.   The imbricated epidermic cells forming the nest
       of the bulb.

h. The bulb itself, with

  a. The external **cover-cells**, granular, fusiform and flattened, with conspicuous oval nuclei.

  β. The **rod-cells**, with spherical nucleus, rod-shaped hyaline peripheral and central processes. These will be better seen on teasing out a section.

i. Nerve-fibres running along the dermis of the papillæ, and branching off to the bulbs.

4. Tease out a section to observe the characters of the cells of the taste-bulbs.

SENSE OF TASTE AND SMELL.

1. Pinching the nose tightly, so as to suspend the sense of smell, attempt with the eyes shut to distinguish by taste between pieces of an apple, an onion, and a potato of similar consistency. They will taste alike; hence the so-called taste of an onion is an excitement of the sense of smell.

2. Wipe the tongue quite dry, and place on the tip a crystal of sugar or on the back a crystal of quinine; neither will be tasted until they are dissolved.

3. Apply with pointed, non-polarizable (Hdb. p. 345) electrodes a constant current to the tongue; a distinct sensation of taste will be felt, of acidity at the anode, of alkalinity at the kathode.

Keeping the electrodes at the same distance, say two or three millimetres, determine the amount of taste-

sensation in different parts of the mouth.   It will be greatest at the tip and back (region of circumvallate papillæ) of the tongue, and edges of the tongue; least or absent in the anterior and middle surface, under surface of the tongue, and lips.   The soft palate will be found to be highly sensitive, the hard palate little or not at all.

4.   Take two pieces of sugar of equal size, and place one on the tip, and the other on the back of the tongue; the sensation will be most acute at the tip.

5.   Place a drop of quinine solution on the tip of the tongue.   It will hardly be tasted.   Place it on the back; it will be tasted at once.

LESSON XXIV.

THE EYE.

A. DISSECTION.

1. Take a fresh eye of an ox or sheep, and note

   a. The transparent **cornea.**

   b. Surrounding and continuous with this, the dirty-white **sclerotic** which forms the outer coat of the rest of the eye. The posterior two-thirds will probably be covered with fat.

   c. The **conjunctiva,** a continuation of the mucous membrane of the eyelids. In taking the eye out of the orbit this membrane is cut through where it passes from the eyelids to the sclerotic. Dissect it forwards in any one place; it will be traceable to the junction of the sclerotic and the cornea. The epithelium is continuous over the cornea, forming the conjunctiva corneæ (cp. B. *a.*).

2. Clear away the fat surrounding the four straight muscles, it will be seen that their tendons form a layer under the conjunctiva of the sclerotic.

3. Cut away the conjunctiva and muscles, and remove the fat around the **optic nerve;** this pierces the sclerotic on one side, and not in the axis of the eye.

4. Cut through the cornea close to its junction with the sclerotic; the anterior chamber of the eye, containing clear, limpid **aqueous humour,** is thus laid bare. Observe the iris with its central aperture through which projects the anterior part of the lens.

5. At a little distance from the cornea, cut through the sclerotic, being careful not to cut too deeply; it will separate easily from the pigmented subjacent **choroid,** except at the junction of the sclerotic with the cornea, and at the entrance of the optic nerve. In other places there is only a loose connection, chiefly by means of blood-vessels. Remove a strip of a few mm. breadth between the optic nerve and the cornea. Note its dark inner surface, or **lamina fusca;** note also in the front part of the choroid, close to the cornea, the pale fibres of the **ciliary muscle** spreading from the junction of the sclerotic and cornea, backwards over the choroid.

6. Carefully pinch up the choroid about half-way between the optic nerve and the cornea with a fine pair of forceps, and snip it through. Underneath it will be seen a thin membrane, the **retina.**

7. Tear away the retina to expose the clear **vitreous humour** which occupies the posterior cavity of the eye.

8. Tilt the eye so that the cornea is underneath; through the vitreous humour can be seen the choroid coat becoming folded longitudinally as it approaches the lens, and so forming the **ciliary processes.**

    The nervous elements of the retina cease at the level of the commencement of the ciliary process. Their termination is marked by an uneven line, the **ora serrata.**

9.   Holding up the choroid and retina, cut them through
as far forward as the ora serrata; it will be seen that
the vitreous humour separates readily from the
retina as far as that line, but in the region beyond
becomes more firmly attached to the ciliary processes.
If an attempt be made to separate them here with
the handle of a scalpel, it will be found that the pars
ciliaris retinæ (or the non-nervous continuation of
the retina in front of the ora serrata) together with
some of the pigment layer of the choroid will come
away with the vitreous humour.

The so-called hyaloid membrane is the outermost
layer of the vitreous humour, and is said to consist of
fine fibres fused together. At the ora serrata it
becomes united to the membrana limitans interna of
the retina.

10.   Turn the eye with the front uppermost, and cut away
the free edge of the iris; make two incisions at right
angles to one another on the surface of the lens, it will
be seen that the lens is covered by a membrane; this
is the anterior part of the **lens capsule.** Carefully
remove the lens, and trace out the limits of the lens
capsule, noting that it forms a complete investment
for the lens.

11.   Gently separate with the handle of a scalpel the
lens capsule from the front part of the ciliary pro-
cesses, and observe that a membrane, the **suspensory
ligament** or **zone of Zinn** passes from the edge of the
capsule to the ciliary processes, of which it forms the
innermost layer, dipping down into their depressions.
On tracing it backwards it will be found to be con-
tinuous with the membrane spoken of above as
adherent to the vitreous humour.

12. Looking into the eye from the front, observe

   a.   The entrance of the optic nerve.

   b.   The blood-vessels running in the retina and radiating out from the entrance of the optic nerve.

   c.   The iridescence, mainly below the entrance of the optic nerve, caused by the irregular reflection of light from the wavy course of the connective-tissue fibres of the choroid. The hexagonal cells of the retina have here no pigment.

13. Separate the rest of the retina from the choroid, and observe that

   a.   The pigment-layer generally adheres rather to the choroid than to the retina.

   b.   Apart from the pigment-layer the retina appears like an expansion of the optic nerve.

   c.   The retina is firmly attached to the choroid at the ora serrata.

14. Take the eye of a rabbit which has been preserved in potassium bichromate, and imbed in spermaceti and castor oil a piece containing the junction of the sclerotic, cornea, and iris. Cut transverse sections so that the arrangement of the several parts can be seen. Observe

   a.   The membrane of Descemet (cf. B *c*) splitting up at the junction of the cornea and sclerotic into fine transparent bundles, some of which curve round towards the iris, the others spreading out fan-like a short distance into the ciliary process and sclerotic. These bundles of fibres form the **ligamentum pectinatium iridis.**

    b.   The **ciliary muscle;** consisting of bands of un-
striped muscle, radiating from the angle of the
iris and cornea;

    (*c.*   The circular muscle of Müller may be seen as the
cross section of a bundle of fibres lying to the
inside of *b*, in the eye of man and of some other
animals, but it is absent in the rabbit.)

    d.   The pigment-layer of the choroid continuing
over the ciliary processes to form the uvea of
the iris.

    e.   The canal of Schlemm (venous sinus) at the
junction of the cornea and sclerotic.

B.   HISTOLOGY.

   Cornea.   Prepare the cornea of a frog or a rabbit as
in Lesson XXII. § 4.   Divide it into two pieces.

1.   Imbed one piece as to give vertical sections ; in these
observe

    a.   The anterior epithelium, or conjunctiva cornea.
In this note

        α.   The external flattened nucleated cells.

        β.   The middle layers of roundish but irregular
cells.

        γ.   The internal single layer of columnar cells
placed perpendicularly.

    b.   The body of the cornea.   Note

        a.   The narrow transparent zone immediately be-
neath the epithelium with very few and small
cells.

        β.   The rows of connective-tissue corpuscles seen
in profile, with numerous branches communi-

cating not only with the cells in the same row,
but also with those below and above.

γ.　The fibrous laminæ between which, for the
most part, the cells are placed.

c.　The **membrane of Descemet** or Demours: a thick
hyaline membrane, with very sharp outlines.

d.　The posterior epithelium, consisting of a single
layer of large nucleated tesselated cells. In this
section they are seen in profile, covering the
membrane of Descemet.

2.　Imbed the other piece to give horizontal sections, and
observe

The branched cornea-corpuscles seen *en face*. (cf.
Lesson V. B § 4.)

Mount the sections in glycerine. They will in time
become darker, and their structure become much
more evident.

Lens.

1.　Boil the lens of ox or dog in sulphuric acid (1 p. c.)
for 10 minutes. Tease out in glycerine. Observe
the long band-like epithelial fibres with serrated
edges.

2.　Make tangential sections of the lens of rabbit
hardened in potassium bichromate : observe the cut
ends of the fibres.

Iris.

'　Cut out a piece of an iris from an eye which has been
preserved in potassium bichromate, and imbed it so
as to cut radial vertical sections. Observe

11—2

a. On the posterior surface the **uvea** or thick layer of pigment epithelium-cells. The pigment is too abundant to allow the outlines of the individual cells to be seen.

b. The cross sections of the bundles of plain muscular fibres forming the **sphincter pupillæ** near the edge of the pupil, and lying just above the pigment-layer.

c. The body of the iris, consisting of blood-vessels united together by a loose connective tissue in which branched pigment-cells are conspicuous. Near the anterior surface is a denser layer, the so-called anterior limiting membrane. The radial muscular fibres (dilatator) may (with difficulty) be seen near the posterior surface.

Retina.

1. Cut away the anterior half of the eye of a mammal immediately after death : avoid dragging the vitreous humour that the retina may not be displaced. Place the posterior half in potassium bichromate for a week, frequently changing the fluid. Remove the retina carefully, carrying with it if possible the pigment-layer, and treat with alcohol. Stain a small piece with hæmatoxylin, imbed it in spermaceti and castor oil, and mount the sections in dammar.

2. Imbed an unstained piece, and mount the sections in glycerine.

3. Remove the retina from an eye still warm to osmic acid. After 6 hours, treat with alcohol. Imbed and mount in glycerine.

In all cases take a small fragment 2 or 3 mm. square

for making sections. If long vertical sections are
made they are very apt to turn over.

In one or other, observe the following structures
from within (anterior surface) to without (posterior
surface).

a.   The **inner limiting membrane**; from this the
     fibres of Müller are seen to start with a broad
     conical foot and to run vertically outwards; they
     may readily be traced as far as *f*.

b.   The **layer of optic fibres.** The nerve-fibres here
     are without medullary sheaths, or rarely possess
     them.

c.   The **ganglionic layer**; this consists of large
     multipolar cells with conspicuous nuclei.

d.   The **molecular layer,** finely granular and opaque
     in appearance.

e.   The **inner nuclear layer,** consisting of two or
     three rows of somewhat large, spherical or oval
     nuclei with nucleoli.

f.   The **fenestrated membrane.**

g.   The **outer nuclear layer.** In this the nuclei are
     spherical or oval, smaller but more numerous
     than *c*.

h.   The **outer limiting membrane,** seen as a sharp
     well defined line.

i.   The **layer of rods and cones.** Both limbs of the
     more numerous rods may be easily seen; the
     cones are recognized with difficulty.

k.   The **pigment of the choroid** with the fine pencil
     of processes enveloping the outer limbs of the
     rods and cones.

3. Tear off a small piece of the retina from the eye of a sheep; probably the pigment-layer of the retina will be left adhering to the choroid. Take a small piece of this pigment-layer, and mount it in glycerine. Observe *en face* the single layer of hexagonal cells, with large pigment-granules.   (See also § 5.)

4. Tease out in water the small piece of retina treated with osmic acid for 24 hours.   Observe

  a. The isolated rods and cones, their broken, laminated, and often curled outer limbs.

  b. Loose nuclei, belonging to the inner or outer nuclear layer.

  c. Isolated nerve-fibres and ganglionic cells.

  d. Portions of the membrane shewing the fibres of Müller projecting, or fragments of the molecular layer with its reticulum of connective tissue.

  Very excellent vertical sections may frequently by accident be found in thus teasing out osmic acid specimens.

5. Remove the retina of a frog immediately after death, and place for 24 hours in osmic acid; tease out in water.   Observe

  a. The large outer limbs of the rods, their striation and transverse lamination.

  b. The pigment-cells, each consisting of a colourless outer part, containing the nucleus, and a coarsely pigmented part, which splits up into a number of fibres, amongst which are imbedded the heads of the rods.

LESSON XXV.

VISION.

Accommodation.

1. Remove very carefully the sclerotic and choroid from a small portion of the posterior surface of the eye of an ox or a sheep. Place the eye in the end of a blackened tube just large enough to hold it, with the cornea outwards.

   On looking through the tube at any object, the inverted image will be seen shining through the retina.

   Or,

   The eye of any small animal, *e. g.* rat, may be used without any preparation, the sclerotic being sufficiently thin to be transparent. It will be found convenient to place the eye, with the cornea downwards, on the stage of a microscope, and to direct light through it by means of the mirror.

2. Standing some feet before a window, close one eye and hold up before the other, at a distance of about six inches, a needle, so that it is at right angles to one of the horizontal bars of the window.

   Look at the window-bar, the needle will appear dim and diffuse.

Look at the needle, the window-bar will appear dim and diffuse.

The eye can accommodate itself for either the needle or the window-bar, but not for both at the same time. The accommodation for the near object is accompanied by a distinct feeling of effort.

3. Facing a window, or a white surface, close one eye and hold a fine needle vertically before the other. At about six inches the needle will be seen distinctly. Bring it nearer the eye, the image will be dim and diffuse, and at the same time larger. The dimness and apparent increase of size are due to **diffusion,** resulting from imperfect accommodation.

Prick a small clean hole in a piece of card, hold it before the eye and again bring the needle close to the eye. It will be seen distinctly at a much smaller distance than before, and at the same time will appear magnified. It will seem larger because the rays, after passing through the aperture, diverge, and so produce a larger image on the retina. Construct a figure to shew this. It will be seen more distinctly because the diffusion circles are cut off.

For a similar reason an object at a distance viewed through the same hole will appear smaller when the eye is accommodated for a nearer object.

4. Prick in a card two small holes close together, so that both are within the diameter of the pupil. Holding the card before one eye (the other being closed) with the holes horizontal, hold a fine needle vertically at a distance of twelve inches. It will be seen distinctly as one when the eye is accommodated for it, but double when accommodation is made either for farther or

nearer objects. Both the double images will be dimmer than the single one.

When a double image appears and

a.   The eye is adjusted for far objects ; stopping the right-hand hole will make the left-hand image disappear.

b.   The eye is adjusted for near objects ; stopping the right-hand hole will make the right-hand image disappear.

 When the eye is exactly accommodated for the object, the rays passing through the two holes are united at the focus on the retina.

 In *a*, the rays fall upon the retina before they are so united, and from the inversion of images on the retina, the actual disappearance of the right-hand image is referred to the left-hand side.

 In *b*, the rays are united, and cross at the focus before they fall on the retina. Stopping the right-hand hole removes the image on the left-hand side of the retina ; and the disappearance is referred to the right-hand side. Construct a figure to shew this.

 When a single image is seen, stopping either hole merely makes the field of vision appear dim.

This is called **Scheiner's experiment.**

5.   The image being one, bring the needle closer to the eye ; at a certain distance it will become double ; this marks the *near* limit of accommodation.

 Fixing the needle on a sheet of paper, walk away while looking at it through the two holes ; at a certain distance it will become double, this marks the *far*

limit of accommodation. This experiment succeeds best with short-sighted people.

Compare the near and far limits of accommodation **as** fixed by looking at a vertical needle through horizontal holes with those fixed by looking at a horizontal needle through holes placed vertically. The results will differ according to the amount of astigmatism **in** the eye.

6. **Helmholtz's Phakoscope.** This should be used in a dark room. The observed looks with one eye through the hole opposite the needle. The observer looks through the hole at the side .A lamp or candle is placed at some little distance from the prisms and shifted about until the observer sees on the eye of the observed, when the latter looks at a distant object, two small bright patches of light on the cornea, two larger but dimmer patches on the anterior surface of the lens, and two small and very dim (not readily seen) patches on the posterior surface of the lens.

Let the observed now accommodate for the needle in front of him, making every effort not to move the eyeball. The observer will see the two patches on the anterior surface of the lens approach each other, while the other two pair remain motionless, thus shewing that during accommodation for near objects the *anterior surface* of the lens becomes more convex.

Observe that in accommodating for near objects the pupil becomes smaller, and in accommodating for far objects the pupil becomes larger.

7. **Astigmatism.** Hold before the eye a card marked with a number of parallel horizontal and vertical lines, and gradually bring the card near to the eye. At a

certain distance both vertical and horizontal lines will appear dim and diffuse, the near limits of accommodation for both having been passed. With care however (with most eyes) a distance may be found at which the horizontal lines are still sharp and well defined, the vertical are already dim and diffuse, the near limit of horizontal accommodation being nearer than that of vertical accommodation.

A similar experiment, especially by short-sighted people, may be tried for the far limit.

Or,

Fix a needle vertically on a board. Looking at the needle with one eye, accommodate the eye exactly for it. Then hold another needle horizontally before the first, and move it backwards and forwards until both needles are seen distinctly at the same time. This will be found to be the case when the needles are at some distance apart.

More exact results are however gained by Scheiner's method (§ 4).

8. **Irradiation.**

Cut out two patches of exactly the same size, of white and of black paper.

Place the white on a sheet of black and the black on a sheet of white paper.

Place them some distance off and adjust the eye so as to throw them a little out of the range of accommodation.

The white patch will appear larger than the black one.

9. **Blind Spot.**

Make a bold mark on a sheet of white paper, place the sheet on the table, and, closing the left eye, fix the axis of vision of the other, by steadfastly looking at the mark at a distance of about 10 inches. Dip a new quill-pen in black ink and place it close to the paper near the mark. Keeping the axis of vision fixed, and the head at the same distance from the table, move the pen outwards (towards the right) from the mark. At a certain distance it will become invisible; mark this spot on the paper. Carry the pen still farther outwards. It will again come visible; mark this spot also. The two spots will indicate the outer and inner limits of the blind spot. Similarly the upper and lower limits may be traced, and with a little practice an outline of the blind spot, shewing even the commencement of the retinal blood-vessels as they emerge from the edge of the optic disc, may be constructed.

The size of the blind spot may be calculated from the formula $\dfrac{f}{F} = \dfrac{d}{D}$, where f is the distance of the eye from the paper, F the distance of the retina from the nodal point of the eye (average = 15 mm.), d the diameter of the outline on the paper, and D the outline of the blind spot.

10. **Region of Distinct Vision.**

Fix the axis of vision by steadfastly looking at some mark. Make on a card two dots so close that they can just be seen as two when placed close to the mark. Keeping the axis fixed, move the card towards the periphery of the field of vision; the dots will soon appear as one.

Or,

Make two dots on a card ·5 mm. from each other. Fix
the axis of vision.   Place the card outside the whole
field of vision and gradually move it inwards to-
wards the mark; the dots will first become visible as
one, and only appear as two as they approach the
centre of the field, and enter the region of distinct
vision.

It will be found on examination that the outline of
this region is not circular but very irregular.

11. Purkinje's Figures.

Go into a dark room with a lighted candle : looking
steadfastly with one eye towards a wall[1], hold the
candle to the side of that eye so that while the eye
is illuminated the image of the candle is not seen,
and gently move the candle up and down.   In a few
seconds the subdued reddish glare caused by the
candle-light will be marked by branching dark lines,
which will be seen to form an exact image of the
retinal vessels as seen with the ophthalmoscope.   The
dark lines are shadows of the blood-vessels, con-
sequently the structures in which the physiological
processes which give rise to the sensation of light
begin must lie behind the retinal blood-vessels.

A round space, in which the blood-vessels are absent,
may with care be seen; this is the yellow spot.

Or,

Turn the eye inwards towards the nose so as to
expose as much as possible of the thin sclerotic

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[1] A light-coloured wall or white blind is the best.   A wall, the paper of
which has any very marked pattern, should be avoided.

behind the cornea. Let an assistant with a lens concentrate the rays of direct sunlight or of a candle or lamp on the sclerotic as far behind the cornea as possible, so that the rays may pass directly through it towards the opposite side of the eye, and gently move the focus to and fro. The same image is still more distinctly seen. The smaller the focus on the sclerotic, the more distinct the image.

If the movement of the light is stopped, the image soon fades away.

In the first method the image moves in the same direction as the light, when the light is moved from side to side, but in an opposite direction when moved up and down.

In the second method the movement of the image is in the same direction as that of the light, whether up and down or from side to side.

12. Look through a microscope with an empty field, illuminated, but not too brightly, by a white cloud, and gently move the head to and fro; an appearance of fine retinal capillaries will be seen as a dark meshwork on a finely punctated ground. In the centre may be seen a spot devoid of capillaries but still finely punctated. If the head be moved from side to side, the vertical meshwork will be seen; if up and down, the horizontal meshwork.

13. **The Yellow Spot. Maxwell's Method.**

Place a moderately strong, but perfectly transparent solution of chrome alum in a flat-sided glass vessel. Resting the eye for a minute or two, suddenly look through the vessel at a white cloud. A rosy spot

or cloud will appear in the centre of vision and re-
main for some little time, but will gradually become
less distinct.

The pigment of the yellow spot absorbs the blue-
green rays between the lines E and F of the spec-
trum, these rays removed from those passing through
the chrome alum, viz. red and greenish blue, leave a
rose colour.

14. Region of normal colour-vision.

Take small pieces of paper of various colours (about
10 mm. square). Fix the axis of vision, on a sheet
of white paper, and place each of the coloured papers
near the axis of vision, they will all be distinct.

Place a red piece near the axis, and then gradually
move it towards the outside of the field of vision; the
redness will vanish and it will appear to be black.

It will be found that passing from the centre towards
the periphery, red is the first to disappear, next
green, and lastly blue. Hence purple becomes violet
and then blue, and rose bluish-white, before dis-
appearing.

15. Positive After-Image.

When waking in the morning, close and shade the
eyes for a minute or two, then suddenly look at the
bright window for a moment or two, and then close
and shade the eyes again. The image of the window
exactly corresponding to the natural one, *i.e.* with
the sashes dark and the panes bright, &c., will last
for some little time.

To succeed, the retina should be in rest beforehand,
and the exposure to the stimulus momentary or
nearly so.

Or, in the evening, having closed and shaded the eyes, suddenly look at a lamp and immediately close the eyes. A similar positive after-image will be seen.

This positive after-image must not be confounded with the negative after-image which comes later. It simply shews that the sensation is of longer duration than the application of the stimulus.

16. Look for an instant at a coloured light, and then look at a white or grey surface, a positive image *of the same colour* will for a brief period be visible.

17. Negative After-Image.

Look fixedly for some time with one eye

a.   At a white patch (*e.g.* white wafer) on a black ground, and then look at a white surface; there will be visible a corresponding dark patch on the white ground.

b.   At a black patch on a white ground, and turn to a grey surface; there will be visible a white patch on a grey ground.

c.   At a red patch, on a white, grey, or black ground, and turn to a white surface; there will be visible a green patch.

And so with the other colours, the colour of the negative image will be complementary to that of the actual object.

d.   Look fixedly at a brightly illuminated window and then close the eye. The *positive* after-image will probably not be seen, in its place

there will come the *negative* after-image with the sashes as bright lines and the panes as dark spaces. This will in turn be succeeded by coloured images.

18. Simultaneous Contrast.

a. Cut out a small piece of grey paper about the size of a shilling, and place it in the middle of a sheet of bright green paper. Cover the whole with a sheet of thin tissue paper. The grey patch will appear pink. The exact tint of the patch will depend on the tint of the green, of which it will be the complementary colour.

Surround the grey patch with a broad dark black rim. The effect of contrast will be lost; the grey patch will appear grey.

On a red ground the grey patch will appear green, and with the other colours similar complementary effects will be produced; but the results are most striking in the case of red and green.

The effect is greatest when the patch is grey, not white, and is always heightened by covering with tissue paper.

b. Cut a thin strip of grey paper and place it across the junction of a red with a green paper, and cover with tissue paper.

The grey will appear green on the red side and pink on the green side.

c. Place a sheet of white paper on a table before a window illuminated by reflection from a white

cloud, not with direct sunlight. On the side of the paper opposite the window place a lighted candle, and between it and the paper place a book edge-ways, or any object which will throw a shadow on the paper. Between the paper and the window place a similar object, throwing a like shadow. The distance of the candle should be such that the two shadows are of nearly equal intensity.

The shadow from the candle, though illuminated by the white sunlight, will appear blue, the complement of the reddish yellow colour of the rest of the paper illuminated by the candle.

This effect of contrast is subjective, not objective. To show this, blow out or place a screen before the candle so as to do away with the candle-light. The place formerly occupied by the shadow now appears white. Through a small black tube, e.g. a piece of black paper rolled up, with so small a bore that the whole field of view lies within the shadow, look at the centre of the area formerly occupied by the shadow. It still of course appears white. While looking, let some one light the candle, or remove the screen. No change will be visible to the observer looking through the tube.

If the tube however be directed partly on the area of the shadow, and partly outside, the blue tint of the shadow will, on the candle being lighted or the screen removed, become apparent as before.

The daylight-shadow heightens the effect on the candle shadow, but may be dispensed with.

In place of sunlight and candle, two coloured lights may be used.

In the above experiments (§§ *a, b, c*) avoid looking at the colours too fixedly and for too long a time. Otherwise the results will be modified by after-images.

LESSON XXVI.

SPINAL CORD.

1. MAKE a transverse section of the hardened cervical
    spinal cord of a rabbit or dog, carrying the section
    through the nerve-roots; stain it with aniline blue
    black or hæmatoxylin, and clear with carbolic acid,
    etc. Observe under objective No. 4 the following
    general features :

    a.   The broad and shallow **anterior fissure.**

    b.   The narrow and deep **posterior fissure.**

    In the rabbit the anterior fissure is not always so
    distinctly shallower than the posterior as it is in
    other animals (*e.g.* ox); and the preparation may be
    distorted in mounting so that the fissures no longer
    appear of their normal relative breadth.

    c.   The **pia mater** surrounding the cord, consisting
    of connective tissue, and having numerous blood-
    vessels running in it.   It will be seen to be
    divided into an outer part, easily separable from
    an inner part which sends into the cord numer-
    ous septa.   The outer part dips down into the
    anterior but not into the posterior fissure.

d.   The entrance into the cord of the **anterior roots** of the spinal nerves in several small bundles.

e.   The entrance into the cord of the **posterior roots** of the spinal nerves in a compact mass.

f.   The **white substance** forming the outer part of the cord and divided on each side by the entrance of the nerve roots into **anterior, lateral** and **posterior columns.**

g.   The division of each posterior column into two by a wedge of connective tissue ; the median smaller part is called the slender fasciculus.

h.   The **grey matter** divided on each side into **anterior** and **posterior cornua,** but with no distinct separating line between them. Note the numerous nerve-cells; they are much more conspicuous in the anterior than in the posterior cornua.

i.   The **central canal;** probably it will be plugged up with epithelial cells ; in the young animal it is lined by a layer of ciliated epithelium.

j.   A layer of connective tissue surrounding the central canal, and

k.   On either side of this, the anterior and posterior **grey commissures.**

l.   The anterior **white commissure** in front of the anterior grey commissure.

m.   A round or oval deeply stained area, forming the hinder part of the posterior cornu, the **substantia gelatinosa.**

2.   Cut tranverse sections of the spinal cord of a rabbit or dog taken from the dorsal and lumbar regions,

treat as in § 1, and compare with that of the cervical region.   Note that

a.   The sections taken from the cervical and lumbar regions are larger than those taken from the dorsal.

b.   The enlargements in these regions are due to an increase in the quantity of grey matter.

c.   The quantity of white matter is greatest in the cervical region, less in the dorsal, and least in the lumbar; that is, the quantity of white matter in the spinal cord increases from below upwards.

d.   In the dorsal region there is a small lateral collection of cells behind and to the outside of the central canal; this is **Clarke's column,** or the posterior vesicular column. It becomes indistinct in the cervical and lower lumbar regions.

e.   Another small group of cells, the tractus intermedio-lateralis, may be seen chiefly in the lower cervical and upper dorsal regions midway between the anterior and posterior cornua.

3.   Take a short piece of the spinal cord of a calf or ox and divide it longitudinally through the anterior and posterior commissures.   Divide one of the pieces so obtained into two through the central canal and lateral columns.   The cord should have been hardened in ammonium bichromate from fifteen to twenty days. Imbed both pieces and cut sections.   Treat as before. Note under objective No. 7 or 8,

a.   The division of the connective-tissue septa into fine fibres forming a fairly regular meshwork, the **neuroglia.**   Blood-vessels can easily be seen running in the thicker parts of the meshwork.

b. The transverse sections of the nerve-fibres occupying the spaces of the meshwork spoken of above. The axis-cylinders will be deeply stained, the medulla forming a clear ring round them. Note their varying size.

c. Surrounding the medulla a clear matrix with a plexus of very fine fibres.

d. The large nerve-cells in the *anterior cornu* with distinct nucleus and nucleolus. They may have two, three, four, or more processes, some of which may be traced for a considerable distance, some divide very rapidly.

e. The collection of the nerve-cells in the anterior cornu more or less distinctly into three groups : a large lateral group, a small median group, and a still smaller anterior group. This is best seen in the cervical and lumbar regions.

f. In the *posterior cornu* the nerve-cells are smaller.

g. The cross section of vertical fibres, both in front and behind the substantia gelatinosa.

h. Throughout the whole of the grey substance, a close plexus of very fine fibres.

Note the following horizontal fibres :

a. Fibres going from the anterior cornu of one side across the anterior white commissure to the anterior columns.

b. Fibres proceeding from the anterior cornu to ascend in the lateral columns.

c. The posterior nerve-roots, entering the cord and some running through the substantia gelatinosa.

    d.   Fibres between the slender fasciculus and the posterior cornua.

4.   Tease out under a low power one of the sections including an anterior cornu; observe in the nerve-cells the single unbranched axis-cylinder process and the branching processes forming a very fine mesh-work of nerve-fibrils, the protoplasmic processes.

5.   Cut an antero-posterior longitudinal section passing through both the anterior and the posterior nerve-roots, staining with aniline blue black.

    Note

    a.   The longitudinal medullated fibres of the anterior column.

    b.   The anterior nerve-roots entering obliquely.

    c.   The nerve-cells and fibres of the anterior cornu.

    d.   The substantia gelatinosa of the posterior cornu, with vertical fibres on either side and curved fibres running through it.

    e.   The longitudinal medullated fibres of the posterior columns, in which can be seen

    f.   The cut ends of the posterior nerve-roots.

LESSON XXVII.

DISSECTION OF THE BRAIN OF A DOG OR SHEEP. *

1. CAREFULLY handling the brain to avoid breaking the nerve-roots, observe

   a. The **cerebrum**, forming the large anterior mass, divided into two hemispheres by a median longitudinal fissure, each hemisphere having on its surface many indentations or sulci, with elevations or convolutions between them.

   b. The **cerebellum**, behind, and partially covered by, the cerebrum, divided into a middle and two lateral lobes, each having slight, and, for the most part, transverse, folds.

   c. The **medulla oblongata**, continuous with the spinal cord. In the middle line are seen the

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* The brain should be removed from the skull as carefully as possible, especial pains being taken to cut the internal carotid arteries and the cranial nerves close to the skull. When the brain is removed it is best to tie the internal carotid arteries, and inject spirit of about 90 p. c. into the basilar artery, and then place the brain in spirit. It may be dissected in a few days, but a longer time is preferable. If the brain be not injected, it should be placed for two or three days in *weak* spirit of about 50 p.c., and then transferred to 90 p. c. spirit. The time required for hardening will in this case be at least a week.

small **posterior pyramids,** and on either side the more conspicuous **restiform bodies.** The former are the forward prolongations of the slender fasciculi, the latter include fibres from all three columns of the spinal cord.

d.   The **pia mater,** a thin membrane closely surrounding the whole of the brain, and dipping down into the sulci. In it run the vessels which supply the brain with blood.

2.   Tearing away the pia mater, turn forward the cerebellum to observe the upper part of the medulla oblongata. Note

a.   The posterior pyramids and restiform bodies diverging, and so forming two sides of a rhomboidal space, the **fourth ventricle.** Trace the restiform bodies forwards; they enter the lateral lobes of the cerebellum, and are called the **inferior peduncles** of the **cerebellum.**

b.   The **choroid plexuses** of the **fourth ventricle** formed by projections of the pia mater.

c.   Having removed the choroid plexuses, the **calamus scriptorius,** or angle formed by the divergence of the posterior pyramids.

d.   The opening into the central canal of the spinal cord, at the point of the calamus scriptorius.

3.   Turn back the cerebellum, tearing away the pia mater which dips down in front of it, and observe

a.   Projecting from the under surface of the cerebrum two round bodies, the **testes.**

b.   Immediately above these, and extending on each side, a cleft, the **transverse fissure.**

4. Gently separate from the testes the pia mater, which
    enters through the transverse fissure to form the
    velum interpositum (see 18), and observe

    a. Immediately in front of the testes two other
        round bodies. These are the **nates,** and, together
        with the testes, form the **corpora quadrigemina.**

    b. Behind the testes are two rounded bands of fibres
        proceeding from the latter to the cerebellum.
        These are the **superior peduncles** of the **cere-
        bellum.**

    c. The **valve of Vieussens**, a thin layer of nervous
        substance stretching between the superior pedun-
        cles, and covering in the anterior part of the
        fourth ventricle. In the anterior part of the
        valve the roots of the fourth nerve may be seen
        rising from the middle line, and curving round to
        reach the base of the brain.

    d. The small aperture in the median line below the
        valve of Vieussens and the corpora quadrigemina,
        and continuous with the anterior part of the
        fourth ventricle, leads to the third ventricle
        (cp § 23).

5. Separate gently the cerebral hemispheres, and note
    the **corpus callosum** or connecting band of transverse
    fibres between them.

6. Note on the under surface of the brain

    a. The medulla oblongata, with two eminences, the
        anterior pyramids, on either side of the median
        longitudinal fissure. Carefully separate with the
        handle of a scalpel the anterior pyramids, and
        observe the decussation of the fibres.

b.   Externally to the anterior pyramids just below
      the pons, two oval elevations, the **olivary bodies.**

c.   The **pons Varolii**, a broad band of transversely
      arranged fibres with a shallow median groove,
      immediately in front of the medulla. This
      connects the lateral lobes of the cerebellum with
      one another, and is called the **middle peduncle** of
      the **cerebellum.** At the lower edge of the pons
      will be seen a narrow 'supplementary' band,
      which passes underneath the anterior pyramids.

d.   The **crura cerebri**, or peduncles of the cerebrum,
      two broad bands of fibres which appear at the
      anterior edge of the pons Varolii, and run for-
      wards diverging from one another.

e.   The posterior perforated space, or space between
      these diverging fibres; it forms part of the floor
      of the third ventricle. When the pia mater
      has been removed it will be found to be pierced
      for the passage of small blood-vessels (to the
      optic thalamus).

f.   The **corpus albicans** or mammillare in front of
      the posterior perforated space. In the dog there
      is a shallow median groove dividing it into two.

g.   Immediately anterior to this the **pituitary body;**
      a round body on a stalk, the **infundibulum,**
      springing from the tuber cinereum which forms
      part of the floor of third ventricle (§ 20 c). The
      pituitary body will probably have been broken
      off in removing the brain from the skull.

h.   The **optic tracts**, two flat bundles of fibres
      coming obliquely forward over the front part of
      the crura cerebri, and meeting in the middle line

to form the optic chiasma. The optic nerves will probably have been cut off.

7.   Looking at one side of a brain of a *dog*, observe

a.   The **Sylvian fissure**, a deep fissure coming up from the under surface of the cerebrum and running backwards and upwards.

b.   The **supra Sylvian convolution**, forming a curve round the posterior part of the Sylvian fissure.

c.   The **inferior external convolution**, forming a fold immediately above (*b*).

d.   The **middle external convolution**; this is nearly straight antero-posteriorly; in the posterior part there is a longitudinal sulcus giving it a bifid appearance.

e.   The **superior external convolution**, bounding on one side the median longitudinal fissure.

It may be noticed that the above general plan of the convolutions is to a certain extent modified in different dogs by secondary sulci, so that in some the division of each half of the cerebrum into four convolutions may not be evident at first sight.

8.   Note on the upper surface of the brain of a dog

a.   The **crucial sulcus**; this is a sulcus at about the anterior third of the brain, running on each side from the median longitudinal fissure outwards in the superior convolution, and bifurcating. A line drawn through it to the end of the Sylvian fissure roughly divides the convolutions into frontal and parietal portions.

  b. The **sigmoid gyrus**; this is that part of the external convolution which bounds the crucial sulcus.

9. Separating the hemispheres, note

  a. The **calloso-marginal convolution,** running above the corpus callosum, and separated above by a fissure from the superior external convolution. This is marked by more or less distinct sulci.

  b. The **hippocampal convolution**; this is the continuation of (*a*) on the under surface of the brain.

10. Take now a fresh brain, reserving the brain used above for reference to any points that do not appear clear on the first dissection. Trace the chief blood-vessels running in the pia mater of the under surface of the brain. At the upper part of the medulla will be seen two arteries. These are the **vertebral arteries,** which having given off recurrent branches, along the anterior fissure of the medulla, curve round to unite in the median line; the vessel formed by their union is called the **basilar artery,** and runs forwards in the median line of the pons Varolii. At the front edge of this it divides into two, called the **posterior cerebral arteries,** each of which running obliquely forward passes to the median side of the third nerve as it springs from the crus cerebri.

Just beyond this each posterior cerebral divides into two branches. One of these runs backward; the other proceeding forwards, is, a little behind the optic commissure, joined by the **internal carotid artery**. Each arterial trunk so formed passes round the optic

commissure, and divides into the **middle cerebral artery** which runs in the fissure of Sylvius, giving off numerous branches, and the **anterior cerebral artery** which passes forwards between the front lobes of the cerebrum, having a large transverse communicating branch with its fellow of the opposite side. The anastomoses between the branches of the posterior cerebral and the internal carotid arteries and those between the two anterior cerebral arteries complete the **circle of Willis.**

11. Carefully remove the pia mater and observe the origin of the nerve-roots.

a. The olfactory nerves, or more correctly olfactory lobes, proceeding from the under surface of the anterior part of the cerebrum.

b. The optic chiasma and tracts (§ 6 *h*).

c. The third pair of nerves, arising from the inner surfaces of the crura cerebri in front of the pons Varolii (§ 10).

d. The fourth pair of nerves, which will be seen curving round the front edge of the pons Varolii; they originate a little behind the corpora quadrigemina (§ 4 *c*).

e. The fifth pair of nerves, large and conspicuous, arising from the sides of the pons Varolii.

f. The sixth pair of nerves, arising in front of the olivary bodies and anterior pyramids close behind the pons.

g. The seventh pair of nerves, arising from the depression between the olivary and restiform bodies close behind the pons.

> *h.* The eighth pair of nerves, arising from the resti-
> form bodies a little to the outside of the seventh
> pair. Sometimes fibres proceed from the seventh
> to the eighth, rendering it difficult to distinguish
> them.
>
> *i.* The ninth, tenth and eleventh pairs of nerves,
> each arising by several roots from the restiform
> bodies, in a line with the eighth but more poste-
> riorly.
>
> *k.* The twelfth pair of nerves, arising between the
> anterior pyramids and the olivary bodies by
> several roots.

12. Slice away the surface of the cerebrum to the level
of the corpus callosum, noting the central white
medullary substance and the thin cortical grey layer.

> In the dog the lateral ventricles project above the level of
> the corpus callosum; when they are reached it is as
> well to proceed at once to lay them bare.

Observe the curving of the corpus callosum in front
and behind.

13. Make a slight incision along each side of the corpus
callosum and so lay bare the **lateral ventricles**; re-
moving the necessary parts of the cerebrum, trace out
the anterior and descending cornua of the ventricles.

Note

> *a.* The **corpus striatum**, a projection into the floor
> of the anterior cornu.
>
> *b.* The **hippocampus major** or **cornu Ammonis**, a
> considerable projection into the descending cornu.
>
> *c.* The **choroid plexus** of the **lateral ventricle**,
> lying immediately in front of (*b*), and continuing

in front as far as the commencement of the anterior cornu.

d.   Between the corpus striatum and the choroid plexus is a ridge which is brought more into view by turning back the choroid plexus. This is part of the **optic thalamus**. It forms a larger part of the floor of the lateral ventricles in the sheep than in the dog.

14. Turn forwards the choroid plexus, it will be seen that it normally overlies a narrow band with a free edge; the part of this band adjoining the hippocampus is called the **corpus fimbriatum.**

15. Holding up the corpus callosum, the lateral ventricles will be seen to be separated by a thin membrane, the **septum lucidum.** It is really a double membrane. Cut through the corpus callosum behind and gently turn it forwards, separating it from the septum lucidum. In the anterior part of the septum lucidum a very small narrow cleft may sometimes be seen; the **fifth ventricle.**

16. Cut away the corpus callosum, and observe underneath it the **body** of the **fornix.** This is a triangular lamina, the narrow anterior part of which dips down just behind the anterior bend of the corpus callosum. Posteriorly it divides into two diverging bundles of fibres, the **posterior pillars** of the **fornix,** part of which spread out over the hippocampus major, the rest forming the corpora fimbriata. This will be rendered clearer on turning back the fornix (§ 18).

17. Trace forwards the choroid plexuses; they will be found to dip down and join under the anterior narrow part of the fornix through the **foramina of Monro.**

18.   Pass the blade of a pair of scissors through the fora-
      mina, and cut through the fornix.   Carefully turn
      back the fornix.   A vascular membrane, the **velum
      interpositum**, will be seen underlying it; this folds
      round the edge of the fornix to form the choroid
      plexuses. Separate the fornix from the velum; the
      diverging posterior pillars will now be well seen; the
      part of the corpus callosum between them is called
      the lyra.   The thickened posterior free edge of the
      corpus callosum forms the upper boundary of the
      transverse fissure.

19.   Turn back now the velum interpositum, and note that
      the foramina of Monro form the anterior part of a
      space, the **third ventricle**, underlying the velum in-
      terpositum and continuous with the lateral ventri-
      cles.   Normally the third ventricle is only continuous
      with the lateral ventricles by the foramina of Monro,
      since the thin epithelial layer of the lateral ventricles
      passes from the corpora striata to the fornix, *over* the
      choroid plexus.   In separating the velum posteriorly,
      note two projections downwards into a median fissure,
      the **choroid plexuses** of the **third ventricle**; be care-
      ful not to injure a round body, the **pineal gland**,
      situated immediately in front of the corpora quadri-
      gemina.

20.   In the surface now exposed, observe

      a.   The **anterior pillars** of the **fornix**, two vertically
           descending cords into which the anterior part of
           the fornix divides.   They descend to the base of
           the brain, and curving round form the external
           part of the corpus albicans.

b.   The **anterior cerebral commissure**, a small white
      bundle of fibres running transversely in front of
      the anterior pillars of the fornix, and seen
      between them. It connects the corpora striata.

c.   The central depression or **third ventricle.**   Cut-
      ting away the necessary part on one side, trace
      the passage from this into the infundibulum.

d.   The **optic thalami** forming the lateral bounda-
      ries of the third ventricle.   Trace the optic tracts
      to these.

e.   Proceeding across the third ventricle connecting
      the optic thalami, a bridge of grey matter, the
      middle cerebral commissure.   This is very
      easily torn through.

f.   On the inner edge of each optic thalamus a
      ridge proceeding to the pineal gland; these are
      the **peduncles of the pineal gland.**

g.   The small, white, **posterior cerebral commissure,**
      in front of, though partially covered by, the
      pineal gland.

21. Cut through the corpus striatum on one side, and
     observe the external layer of grey substance, and the
     central striated appearance from fibres running in
     from the crus cerebri.

22. Between the optic tracts and the corpora quadri-
     gemina, note two oval bodies, the **corpora geniculata,**
     and the band of fibres on either side proceeding from
     the nates, and dipping down under the corpus genicu-
     latum internum to join the optic tracts.

                                              13—2

23. Trace out the passage from the third to the fourth ventricle, or **aqueduct of Sylvius.** It will be found to pass beneath the posterior cerebral commissure, and the corpora quadrigemina.

24. Divide the cerebellum by a longitudinal vertical incision in the median plane; observe the arborescent appearance (*arbor vitæ*) produced by the arrangement of the outer grey and inner white substance. Make a similar longitudinal section through the middle of one of the lateral lobes; note in the midst of the white substance the irregular outline of the grey matter of the **corpus dentatum.**

25. Divide a sheep's or a dog's brain in half, by a longitudinal section carried carefully through the median line, and observe

The corpus callosum, with its anterior and posterior bend, the latter being continuous with the fornix, the body of which is seen as a thin layer lying beneath the corpus callosum and curving downwards anteriorly towards the corpus albicans as the anterior pillars. Since these diverge, they will be visible only in part of their course.

The septum lucidum and the lateral ventricle.

The transverse fissure; the velum interpositum entering the transverse fissure, underlying the fornix and forming the roof of the third ventricle.

The aqueduct of Sylvius; the valve of Vieussens passing from the corpora quadrigemina and entering into the median lobe of the cerebellum.

The pineal gland with its peduncle.

The posterior commissure of the cerebrum: it is covered by a thin lamina formed by the united peduncles of the pineal gland. In the section this lamina appears as a band proceeding from that body. The large middle commissure, forming a considerable portion of the cavity of the third ventricle. The anterior commissure, a little below the apparent termination of the anterior pillar of the fornix.

The optic tract.

The pons Varolii.

LESSON XXVIII.

DISSECTION OF THE LARYNX.

1. IT is preferable to obtain a fresh larynx of a sheep or an ox from the butcher's; but the spirit-preserved larynx of the dog (Lesson I.) will serve. The larynx will probably be obtained with the upper part of the œsophagus attached, and surrounded by a mass of muscle and connective tissue.

Having slit up the œsophagus lengthways, turn back or cut away the sides and observe the opening into the larynx bounded in front by the epiglottis, at the sides by folds of the mucous membrane, and behind by the large converging yellow crests of the arytenoid cartilages. On looking down into the larynx the opening between the vocal cords, or **rima glottidis,** may be seen at some depth below. Observe that the mucous membrane of the œsophagus is continuous with that of the larynx. Bend down the epiglottis projecting from the front, upper, edge of the larynx, and note that the passage to the larynx is then quite closed.

2. From the posterior surface carefully remove the œsophagus with the pharyngeal muscles. From the

sides dissect away the sterno-thyroid muscle (Lesson I.
§ 24), taking care not to injure the muscle lying
underneath it (§ 3), and clear away the whole of the
thyro-hyoid muscle which covers the side of the
thyroid.   The hyoid bone and thyro-hyoidean mem-
brane may be left.   The outlines of the thyroid will
now come into view, clear away the connective tissue
until they are quite distinct.   Note that

The **thyroid cartilage** consists of two lateral laminæ,
which meet in front and diverge behind, and have
their upper and lower posterior angles prolonged,
forming the upper and lower cornua.   Observe in
front the rounded projection or Adam's apple.

3.   Observe the **crico-thyroid muscle** spoken of above (§ 2);
dissect it away, noting its attachments.   The **cricoid
cartilage** will come into view, and it will be seen
that the muscle in question passes from the posterior
cornu and posterior portion of the lower margin of the
thyroid, to the front part of the cricoid.   Observe
that below this muscle there is stretching between
the two cartilages a membrane which limits the move-
ments of the thyroid.

4.   Observe the articulations of the posterior cornua of
the thyroid to the cricoid.   Disarticulate one cornu,
cut through the crico-thyroid membrane, and remove
one half of the thyroid, taking care not to injure any
of the muscles. Trace out with the finger the outlines
of the cricoid cartilage.   Note that

It forms a complete ring, which in front is narrow,
and is covered by the edge of the thyroid, but behind
is deep and enclosed on either side by the thyroid.

5. On the posterior surface of the cricoid note on either
    side of a central ridge two laminæ of muscle, the **pos-
    terior crico-arytenoids;** cut through their attach-
    ment to the cricoid, and reflecting them from below
    upwards note that they are attached above to the
    external angles of two cartilages, the **arytenoid
    cartilages,** placed on the posterior upper edge of the
    cricoid cartilage.

6. Carefully clear away the tissue from the back of the
    arytenoid cartilages above the cricoid, and observe on
    the posterior surface of the former the **arytenoid
    muscle;** cutting it through the middle, which in the
    sheep is frequently tendinous, reflect it on either
    side; it will be seen to be attached to the back of
    each arytenoid cartilage; the posterior surface of the
    arytenoid cartilage will now be laid bare, and its
    articulation with the cricoid cartilage can be made
    out.

7. Looking at the larynx where the thyroid has been cut
    away, observe the **lateral crico-arytenoid muscle.**
    Cut away its attachment to the lateral portion of the
    upper margin of the cricoid, and, reflecting it, observe
    that it passes backwards and upwards from the cricoid
    to be inserted into the arytenoid cartilage just in
    front of the insertion of the posterior crico-arytenoid.

8. Clearing away the fat and connective tissue from
    the side of the larynx, observe the **thyro-arytenoid
    muscle** stretching across from the thyroid in front
    to the arytenoid behind. Cut it through in the
    middle and reflect both ends. Note its origin from
    the angle of the thyroid and its insertion into the

lateral surface of the arytenoid in front of the insertion of the lateral crico-arytenoid.

Remove the muscle altogether, and observe the lateral surface of the arytenoid cartilage.

9. Cut away on the same side the underlying mucous membrane; the interior of the larynx will now be laid open.   On the opposite side the indistinct vocal cord will be seen passing as a pale band of tissue from the anterior angle of the arytenoid cartilage to the angle of the thyroid. The inner or medium surfaces of the arytenoid cartilages will be seen to bound a large oval space, called the respiratory space.

10. Clear away on one side any muscle or connective tissue still attached to the arytenoid cartilage, and observe more fully its shape, noting particularly, the anterior projection or processus vocalis, the posterior lateral projection or processus muscularis, and the articulation with the cricoid.

11. Dissect *from the inside* the thyro-arytenoid muscle of the opposite side, and observe more carefully its attachments (§ 8).

The larynx of the sheep differs materially from that of man, by the indistinctness of the vocal cords, by the absence of the false vocal cords and ventricles of the larynx, and by the peculiar crested conformation of the arytenoid cartilages.

DEMONSTRATION.

The use of the laryngoscope.

LESSON XXIX.

TISSUES OF REPRODUCTION.

A. OVARY.

1. Prepare sections of mammalian ovary, hardened in chromic acid or in potassium bichromate. Let some of the sections be longitudinal, and others transverse. Be careful that the sections are especially thin at the periphery of the organ. Stain some of the sections and mount in Canada balsam; mount others unstained in glycerine.

    Examine with No. 3 or 4 objective and observe

    a. The fibrous 'medullary' portion or **stroma**, radiating from the **hilus**, and forming the greater portion of the organ, especially in the case of young animals.

    b. The cortical layer, consisting of small **Graaffian follicles**, and forming a zone immediately underneath the fibrous investment or *tunica albuginea* along the whole periphery except at the hilus.

    c. Larger (older) Graaffian follicles which have sunk into the substance of the stroma, and are thus at some distance from the surface.

Most of the larger Graaffian follicles will be empty; in the moderate-sized or smaller ones may be seen a transparent nuclear-looking body, the **ovum.** Do not confound a small Graaffian follicle with an ovum.

d.   If the ovary be not that of a young animal a considerable portion of the stroma may be taken up by a **corpus luteum.** This will appear as a circular mass of tissue more opaque than the rest of the stroma, frequently containing pigment and exhibiting a radiating structure. The appearances however of the corpora lutea differ according to their age.

2.   Examine, with a high power, the cortical layer. If the sections already prepared are not sufficiently thin, prepare others taken through the cortical substance only. Observe, passing from the surface inwards,

a.   The superficial epithelium (**germinal epithelium**) consisting of a single layer of short columnar cells.

b.   Immediately beneath this, a thin fibrous layer, the **tunica albuginea,** with the bundles of connective tissue arranged both longitudinally and transversely. The lower portions of this pass gradually into the stroma.

c.   The **stroma,** having a fibrous appearance, but more irregular than *b,* and largely composed of spindle-shaped cells.

d.   The smaller Graaffian follicles. In these will be seen

a.   The **ovum,** a spherical and transparent mass of protoplasm, containing some fat granules.

It has no distinct cell-wall. Within it lies a comparatively large, also transparent nucleus, the **germinal vesicle.**

β. A layer of columnar epithelium immediately surrounding the ovum and lining the follicle; this is the **membrana granulosa.**

e. The larger Graaffian follicles. In these will be seen

α. A fibrous investment of the follicle : the tunica or theca follicularis.

β. A lining of epithelium more than one cell deep.

γ. At one spot this epithelium is collected into a mass, the **cumulus proligerus,** in the midst of which is embedded

δ. The ovum, with its cell-substance or vitellus more distinctly granular, and its germinal vesicle marked with a nucleolar looking spot, the germinal spot. In these follicles the ovum has a distinct investing membrane, which in some is much thickened, so as to form a *zona pellucida.* From this the vitellus frequently shrinks in the process of hardening. The cells of the cumulus which lie next to this membrane are frequently arranged in a radiating manner.

ε. The rest of the follicle is filled with fluid.

3. Tease out in weak glycerine a thin and small section of the more superficial stroma. Observe, intermingled with the ordinary connective tissue and blood-vessels, the abundant spindle-shaped fibres.

4. Tease out in weak glycerine a thin section taken from the centre of a corpus luteum. Observe

    a. Cells similar in character to epithelium-cells, often large and loaded with pigment and fatty granules. (Crystals of hæmatoidin may be seen.)

    b. Cells similar to white-blood or lymph-corpuscles.

    c. Spindle and other shaped cells: developing connective tissue.

    The appearances will vary much according to the age of the corpus luteum.

5. In some of the sections taken near the hilus there may be seen (dog, cat) lying in the stroma groups of tubules, lined with a short cubical or flattened epithelium, and cut at various angles. These are the tubules of the **parovarium.**

6. Take the fresh ovary of a sheep or large dog; observe the bulgings due to the more or less ripe Graaffian follicles. Holding the ovary over a glass slide, carefully prick the most prominent follicle and receive the contents on a glass slide. Examine *without a cover slip,* with simple lens or low objective. If the ovum is present it will at once be recognized. When found carefully cover with a cover slip, inserting a ring of thin paper in order to *avoid pressure,* and examine with a higher power. Observe

    a. The thick zona pellucida, with double contour (and radiating striation).

    b. The granular vitellus.

    c.   The transparent germinal vesicle, with its germinal spot. If the follicle be quite ripe these may have disappeared.

    d.   The cells of the cumulus proligerus attached all round the zona pellucida.

B. TESTIS.

    1.   Take a small mammalian testis, *e.g.* of guinea-pig or rat, which has been hardened in Müller's fluid, and cut longitudinal sections through the testis and globus major of the epididymis. Observe with objective No. 3 or 4.

    a.   The **tunica vaginalis propria,** which is round the greater part of the testis firmly connected with the underlying tissue, and is hence called the tunica adnata. It separates from the testis near the epididymis.

    b.   The **tunica albuginea,** the thick investment proper of the testis; near the epididymis it is very much thickened, forming the **corpus Highmori;** towards this radiate bands of connective tissue from the rest of the albuginea, forming the septula testis and so dividing it into lobules.

    c.   The **tubuli seminiferi,** contorted and anastomosing tubes occupying the lobular spaces; near the corpus Highmori the tubes unite, and the tubes so formed, the **vasa recta,** have a short, tolerably straight course; in the corpus Highmori they anastomose with one another, forming the **rete testis;** from the outer edge of this may be seen one or two larger tubes, the **vasa efferentia,**

which originate from the rete and open into a
still larger tube, the canal of the **epididymis.**

2. Cut thin sections of the lobular part of the testis
of a dog, and observe with a high power

a. The tunica vaginalis, consisting of fine fibrous
connective tissue, having externally a single
layer of tessellated epithelium.

b. The tunica albuginea, of coarser connective tissue,
with numerous blood-vessels running in it, and in
the septula testis.

c. The intermediate tissue between the tubuli
seminiferi. In the dog this is small in amount,
and consists mainly of fine connective tissue con-
taining numerous connective-tissue corpuscles,
and of blood-capillaries.

d. The tubuli seminiferi. In these note

α. A tunica adventitia of elongated and branch-
ing cells.

β. A membrana propria.

γ. The cells filling the tubes, to a greater or less
extent, so that there is no distinct lumen.
They are for the most part polyhedral from
compression, with large nuclei staining deeply
with hæmatoxylin, and small nucleoli. Other
forms of cell may be seen.

δ. The spermatozoa, lying generally in bunches;
the heads staining deeply. The method of
development of these from the cells in the
seminal tubes is still a matter of dispute.

3. Cut sections of the lobular part of the testis of a pig, cat, or rat, and observe that

The tissue surrounding the tubuli seminiferi or inter-mediate tissue differs from the corresponding tissue of the dog's testis in containing a large number of polyhedral cells with round nuclei, not unlike hepatic cells.

4. Tease out in water a small fragment from a piece of testis that has been treated with osmic acid 1 p. c. for twenty-four hours, and observe more particularly the characters of the cells.

5. Cut a section through the corpus Highmori, and observe that

a. The tubuli form a network of passages without distinct walls.

b. The passages are lined with tabular or cubical epithelium.

6. Cut a transverse section through the globus major of the epididymis, and note both in the vasa efferentia and in the canal of the epididymis,

a. The external fibrous coat.

b. The circular muscular coat.

c. The basement membrane.

d. The epithelium, consisting of cylindrical ciliated cells, with two or more rows of small cells between them and the basement membrane. In the canal of the epididymis the cylindrical cells are long and slender.

     e.   The distinct lumen.

     f.   The mass of spermatozoa, occupying in some cases the lumen.

7.   Cut a transverse section of the vas deferens.   Note

     a.   The external fibrous coat.

     b.   The muscular coat of three layers, two longitudinal and one circular, the latter being the most developed.

     c.   The ciliated columnar epithelium.

8.   Cut in half the fresh testis of a rat, and gently press the cut surface on a glass slide. Observe the spermatozoa, each consisting of

     a.   An ovoid head.

     b.   A long tapering tail.

     c.   A short intermediate part.

Note that the spermatozoa move by a whip-like movement of their tails.

9.   Observe in like manner the spermatozoa of a frog. In Rana temporaria there is no head, but only a long thread of protoplasm.

APPENDIX.

NOTES ON THE USE OF THE MICROSCOPE[1].

See that the tube of the microscope moves easily, but not too easily, up and down. It should be occasionally rubbed round with a little olive-oil, and the oil then wiped off with a dry cloth.

With a piece of chamois leather or silk, remove any dust that may be on the lenses of the eye-piece or on the lower lens of the objective. If by accident the objective has been smeared with glycerine, stream it with water from a wash-bottle, and wipe it dry with chamois leather: if with Canada balsam, place on it a drop of chloroform, and rub gently, repeating till the balsam is quite removed: this must be done very cautiously, since the lenses are sometimes fixed in with Canada balsam.

All *unnecessary* rubbing of lenses however should be avoided; hence take care to put them away after they have been used.

It is advisable for the student not to unscrew the separate lenses of a high objective; when however the dimness of an object under observation renders it necessary to do so, care must be taken not to remove the black coating

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[1] The microscope to which reference is made in the text is Hartnack's Stativ III. with oculars 2 or 4 and objectives 3 or 4 and 7 or 8. Nearly corresponding to this on Zeiss' model is Stativ III^b. no. 20 with oculars 2 and 4, objectives A and D.

14—2

on the inside of the tube in cleaning the lenses, and when cleaned to restore them in proper order, to their previous positions.

With No. 4 objective, twist down the microscope-tube till it is about a quarter of an inch from the object ; then looking through the eye-piece, twist the tube *upwards* till the specimen is in focus.

With objective No. 7, lower the tube till it is $\frac{1}{8}$ of an inch from the object, and then slowly twist the tube down, moving the slide about, till the object just becomes visible, then focus by means of the fine adjustment.

Direct sunlight must not be used to illuminate the field; in selecting a permanent position to work with the microscope, it is best, if possible, to face the north,—or if this is not practicable, to have the light on the left-hand side.

With high powers, use the corresponding small diaphragm ; otherwise although the field may look brighter, the outlines of the cells, etc. will not be so well defined.

The student should accustom himself to keep *both* eyes open when using the microscope, and to use *either* the right or the left eye for looking at specimens. The fatigue of microscope work is thus very much lessened. It generally requires a little practice to keep both eyes open, but a few minutes trial every time that the microscope is used will soon overcome the difficulty.

When an object appears dim, it may be caused by some fault in the specimen itself, or by the object-glass not being clean. In the latter case the dimness remains, whether the object is in focus or not. Dust upon the eye-piece can be recognized by the outlines of the particles appearing well defined ; they do not look dim and diffuse.

OBSERVATION OF FRESH TISSUES.

There are but few of the tissues of the body, which in the

fresh state are sufficiently consistent and firm to allow thin sections to be cut with the razor.

This, however, is of no very great importance, since the main end of observing the fresh tissue is to ascertain to what extent the various hardening and staining agents, which enable one or another constituent part of the tissue to be clearly seen, alter the tissue components. By taking a small piece of a tissue, and teasing it out carefully in a normal solution, enough can generally be gathered of the normal appearances to judge whether the reagents used cause any structural modification, or simply bring more clearly to view already existing outlines.

With tolerably firm bodies, *e.g.* the kidney, it is best to cut as thin sections as possible with a razor, either not moistened at all, or covered with normal saline solution, and observe one section under the microscope, mounted in some normal solution, whilst another is carefully teased out.

With structures too yielding to cut sections, a piece should either be snipped off, as with the villi of the small intestine, with a fine pair of scissors, or a strip torn off with a forceps, as with the coat of an artery, and then teased out.

There are certain fluids which, when fresh, cause very slight changes in the tissues; they more or less resemble the fluids with which the tissues in the body are surrounded.

These **normal fluids** are

      a. The aqueous humour of the eye.

      b. Blood-serum.

If, then, a fresh tissue is to be observed from *e.g.* a rabbit, the corneæ should be punctured to obtain the aqueous humour, or the blood should be allowed to clot in a flat vessel, to obtain the serum. In one of these fluids the tissues should be mounted.

   c.   Normal saline solution.

This may be prepared by dissolving 7.5 grm. of sodic chloride
   in 1000 cc. of distilled water.

   d.   Iodised serum.

Iodine is sometimes added to serum to preserve it, so that
   it may be at hand when required ; serum thus iodized is,
   however, far less a 'normal' fluid than the others.   It may be
   prepared by dropping a few crystals of iodine into fresh
   serum, and shaking occasionally.   The fluid should be of a
   light brown colour.

Teasing.

Be careful to take a *small* piece only: it should be viewed
   with a low power before teasing, to ascertain the general
   relation of the parts, and hence to guide the teasing :
   thus, if it consists of parallel fibres, with one needle fix
   the piece at one corner and draw the other needle through
   it in the direction of the fibres ; in teasing out to show
   special objects, *e.g.* ganglion-cells, the arrangement of the
   parts should be *particularly* noticed under a low power.

In teasing, it is important to place the slide on an appropriate
   ground ; if the object is clear, the slide should be placed on
   a piece of black paper, if it is dark, on a piece of white
   paper.   Stained sections should also be teased out against
   a white or grey ground.

DISSOCIATING FLUIDS.

These are fluids which, whilst preserving certain parts of
   a tissue, dissolve or partially dissolve others, so that the
   former can be isolated by teasing or shaking.   As a rule
   the piece of tissue so treated should be only a few mm.
   square.

Weak iodized serum of a light brown colour is in most cases
   the best.   As the tissue absorbs the iodine, a little strongly

iodized serum should be added; or the fluid renewed. The tissue may often be teased out after one day; but a longer time may be necessary.

Chromic acid ·02 p. c. is useful for nerve-cells in spinal cord.

Müller's fluid answers well for olfactory mucous membrane, stomach, kidney.

Weak spirit 36 p. c. is frequently used for isolating epithelial cells.

Baryta water, for tendons, &c.

Osmic acid ·1 to 1 p. c. is a dissociating fluid of general application; it has the advantage of altering the normal appearances very slightly.

There are various other dissociating fluids, but the above will be sufficient to illustrate their use to the beginner.

HARDENING AND HARDENING AGENTS.

General Directions.

The tissue should be removed from the body to the hardening agent as soon as possible after the death of the animal.

Be careful to divide the tissue to be hardened into *small* pieces before placing in the hardening fluid. As a rule the pieces should not be more than 3 to 4 mm. square.

In all cases where a tissue is put in a fluid to harden or have lime-salts extracted from it, the fluid in which it is placed should be relatively abundant, *e. g.* 15 to 20 times the volume of the tissue; it should be renewed after a time varying from 12 to 24 hours.

After the substance has been hardened it should be transferred to *weak* alcohol, *e.g.* 50 p.c. and there be left for two days.

It should then be placed in a little stronger alcohol, *e. g.* 70 p.c., and left for one day; and finally placed in 90 p.c. till required for sections.

This method answers fairly in most cases, but it is more satis-
factory, especially with chromic-acid specimens, to change
the 50 p.c. alcohol every day, till on being left for twenty-
four hours the alcohol remains uncoloured. Tissues per-
meated with chromic acid are not stained by carmine or
hæmatoxylin; moreover chromic acid is not soluble or but
very slightly in strong alcohol, hence the repeated change
of the 50 p.c. In particular cases the tissues should be
transferred from 90 p.c. to absolute alcohol.

The tissues should be placed in a flat short bottle to harden,
rather than a narrow high one; and they must be kept
cool, especially when they are first removed from the body
to a hardening agent.

Preparation of Solutions.

In making solutions containing a definite per centage of
hardening or other substance, remember that 1 cc. of
water at $4°$ C. and 760 mm. pressure weighs exactly 1 grm.,
and that under the ordinary variations of temperature
and pressure it may be taken always to weigh 1 grm.;
a 5 per cent solution of (e.g.) ammonium bichromate
may therefore be made by dissolving 5 grm. of the salt
in 95 cc. i.e. 95 grm. of distilled water, the resulting
mixture must weigh 100 grm. of which 5 are ammonium
bichromate, hence it is a .5 p.c. solution; and a ·5 solution
of gold chloride by dissolving ·5 grm. of the salt in 99·5
cc. of distilled water.

Since it is not important to have the solution *exactly* 5 p.c.
or ·5 p.c., it is usual to take 100 cc. of water in the above
and other similar cases.

A solution of any given percentage can easily be diluted
to one of a lower percentage: thus, if it is required to
dilute a 5 p.c. solution of ammonium bichromate to a
2 p.c. solution; 100 parts of the 2 p.c. solution contain 2
parts of the salt, therefore 250 parts of the 2 p.c. solution

contain 5 parts of the salt, but 100 parts of the 5 p. c. solution contain 5 parts of the salt, henceby diluting the 100 to 250 parts the 2 p. c. solution is obtained.

Chromic Acid.

Dissolve 10 grm. in 1000 cc. of distilled water.

The ·5 solution is that most frequently used; it is easily prepared at any time from the above 1 p. c. solution by diluting with an equal volume of water.

For acting on bone and teeth, 1 cc. of nitric acid may be added to the above; the action is much more rapid, but care must be taken to transfer to alcohol as soon as the salts are extracted.

Chromic acid hardens the tissues by an action analogous to tanning; but renders them, if they are left in it too long, brittle and friable.

Potassium Bichromate.

The 1 p. c. solution is the most useful. Dissolve 10 grm. of the salt in 1000 cc. distilled water.

Ammonium Bichromate.

Dissolve 50 grm. ammonium bichromate in 1000 cc. distilled water. This gives a 5 p. c. solution.

Müller's fluid.

Dissolve 25 grms. of potassic bichromate and 10 grms. of sodic sulphate in 1000 cc. of water.

Picric acid. (Kleinenberg's method.)

Make a cold saturated solution of picric acid, and to 100 cc. of this add 2 cc. of concentrated sulphuric acid; filter, and to the filtrate add 300 cc. of distilled water.

Tissues should be left in this for a comparatively short time, varying from three hours to ten, with the exception of bone, which requires some days, since there are lime salts to be extracted.

Osmic acid.

A 1 p. c. solution of this is the most useful. To avoid reduction of the osmic acid great care should be taken to obtain *pure* water. The bottle should be washed out with sulphuric acid and then with distilled water. If the osmic acid turns dark it must be covered with black paper or kept in the dark.

Most tissues are sufficiently hardened by a stay of twenty-four hours in 1 p. c. osmic acid to allow of sections being at once cut. If not sufficiently hardened, but deeply enough stained, they should be transferred to spirit.

In the presence of organic matter osmic acid is reduced in the light. This takes place quickly in the presence of fatty substances, so that in a tissue which has been stained with osmic acid, the medullated nerve-fibres can be easily traced.

Alcohol.

Alcohol, besides its general use of completing the hardening begun by other fluids, is sometimes used alone. It coagulates the albumin in tissues and then renders them more opaque. Absolute alcohol gives the best results.

IMBEDDING.

Take a *small* piece of tissue only to imbed; of course this cannot be done in many cases, where it is desired to make out the general relation of the parts of an organ, *e.g.* the eye.

Before imbedding press the piece of tissue very gently between blotting paper, to remove as much as possible of the fluid in which it has been placed. Spirit and the other hardening agents, if not removed, cool the imbedding mixture immediately round the tissue, and not mixing with it, prevent any adherence between the mixture and the tissue: with spirit specimens the excess of fluid should not be removed till the imbedding substance, etc. is ready, since the spirit rapidly evaporates, and the tissue dries.

If it is desired that the imbedding substance should not only adhere to the outside of the tissue so as to form a support for cutting, but should also penetrate its spaces and render it firm to cut right through, it should be placed in absolute alcohol for about five minutes to remove all water, and transferred for about half an hour to oil of bergamot. It should then be imbedded in spermaceti and castor oil, with which the oil of bergamot mixes. To cause a thorough filling up of cavities it is advisable to soak the tissue, removed from the oil of bergamot, in the just melted imbedding mixture for a minute or two before imbedding.

For the method of imbedding see Lesson IV. § 4. Note also that

Instead of using a block of imbedding mixture, the following method will frequently be found useful. Cut a small oblong piece of tolerably stiff paper from the angles toward the centre, fold the sides and fasten them together with sealing-wax, so that a small box open above is obtained. Pour the melting imbedding mixture into this $\frac{1}{3}$ of an inch in depth and allow it to cool. The tissue to be imbedded can then be placed on this and the mixture poured over it.

The imbedding substance should be heated as little as possible above its melting point. To keep it at a constant temperature, a sand-bath heated by a small gas jet may with advantage be used.

When the tissue is moved about to remove air-bubbles, etc. in the still liquid imbedding substance, by means of a heated needle, bring down the temperature of the needle to that of the substance by moving the needle about in it before touching the tissue.

Before cutting sections, cut away as much of the imbedding substance around the tissue as possible without causing the tissue to become loose. It is easier to cut thin sections when the surface to be cut is small than when it is large.

The substance in which different tissues should be imbedded
varies with the consistency of the tissue. As a rule the
imbedding substance should be of about the same degree
of hardness as the tissue. Loose tissues with many cavi-
ties must generally, in order to obtain satisfactory sections,
be imbedded in a material which will penetrate the tissue
and fill up the cavities, *e.g.* spermaceti and castor oil:
in this case the sections are usually of little value except
for teasing out, unless the imbedding mixture be removed
by carbolic acid and turpentine.

In some cases thin sections may be obtained which may be
mounted in glycerine by carefully removing the excess of
fluid from the outside of the tissue, and then imbedding
it in a mixture which has but little penetrating power,
not moving it about to remove air-bubbles.

If the tissue has been stained before imbedding, and it is in-
tended to clear it in carbolic acid and turpentine, and mount
in Canada balsam, the section should be cut with a razor
wetted with *olive oil;* in other cases the razor should be
wetted with alcohol.

In preparing the following mixtures, the constituents should
be placed in a capsule on a sand-bath and kept at a tem-
perature just above melting point for an hour or more, the
liquid being occasionally stirred.

Paraffin A.

> Solid paraffin   15 grms.
> Liquid paraffin 15 cc.

This is used to surround the cover-slip in observing the
amœboid movements of white blood-corpuscles, on the warm
stage. It begins to melt at about 37° C., and thus serves
as an indication of the limit of temperature that can be
used in the observation without injury.

Paraffin B.

> Solid paraffin 30 grm.
> Cocoa butter 10 grm.
> Hog's lard   30 grm.

This is a soft imbedding mixture, melting at a low tempera-
ture, and not dissolved by carbolic acid and turpentine. It
does not easily penetrate tissues.

Paraffin C.

> Solid paraffin   30 grms.
> Cocoa butter   20 grms.
> Spermaceti   10 grms.

This is a moderately hard imbedding mixture, soluble in
carbolic acid and turpentine.

Paraffin D.

> Solid paraffin   20 grms.
> Cocoa butter   10 grms.
> Spermaceti   10 grms.

This is a harder mixture, also soluble in carbolic acid and
turpentine.

Spermaceti and Castor Oil.

> Spermaceti 40 grm.
> Castor oil   10 grm.

[An excellent mixture is the following:

> Paraffin 2 parts,
> Vaseline 1 part.

This is transparent enough to enable the position of the
imbedded tissue to be made out; it penetrates tissues well,
is consistent, cuts easily, and is readily soluble in carbolic
acid and turpentine.

If a harder imbedding material is required a little more
paraffin may be added.]

STAINING.

Hæmatoxylin.   (Prepared according to Kleinenberg.)

1.   Take crystallized calcium chloride and add it in excess to
70 per cent. alcohol; shake well and let it stand.   Draw off
the saturated solution and add alum to excess, shake up
and let stand.   Filter.

2. Make a saturated solution of alum in 70 per cent. alcohol by the method above given for calcium chloride. Add this to the filtrate in 1 in the proportion of 8 to 1.

3. To the mixture thus obtained, add drop by drop a saturated solution of hæmatoxylin in absolute alcohol, till it is a *moderately* dark purple. In dissolving the hæmatoxylin in absolute alcohol remember that it is very soluble, and use as little as possible of the spirit.

The solution will become considerably darker after some days.

It may be diluted to any extent required with the mixture of (1) and (2), but as a rule a tolerably quick staining is preferable. If a section is placed in hæmatoxylin for some time it must of course be covered up, or the spirit will evaporate and the solution become concentrated. In many cases it is well to stain a piece of tissue as a whole in hæmatoxylin before imbedding; a tolerably porous tissue naturally serves best for this method. A small piece only should be taken.

If a section which it is wished to preserve has been stained too deeply, the colour may be extracted by placing the section in dilute acetic acid. At the same time it must be remembered that the acetic acid will exert its own proper action on the tissue.

The hæmatoxylin after it has been used should be put aside in a separate bottle, since after filtration it may be used again.

Carmine. (Frey's method.)

a.

Carmine	0·3	grm.
Glycerine	30·0	,,
Alcohol	4·0	,,
Distilled water	30·0	,,
Ammonia	q. s.	

Add the distilled water to the carmine, and then add dilute ammonia drop by drop until the carmine is dissolved,

taking care that the least possible excess is added. If the solution smells strongly of ammonia expose it in a flat vessel to the sunlight for a day or two. Finally add the glycerine and alcohol, and shake. It should be kept in a stoppered bottle, since otherwise the ammonia evaporates and the carmine is precipitated.

It may be diluted with water to any extent required.

For staining fresh tissue the carmine may be prepared thus :

b. Mix in a mortar 1 grm. of carmine with a little water into a thin paste ; add ammonia, drop by drop, till the whole is lake-red, then add 100 cc. of water, and evaporate on a water-bath till the excess of ammonia is evaporated off, and the carmine begins to be precipitated: filter. The excess of ammonia must be got rid of, otherwise the staining becomes diffuse.

This solution will not keep long, and fungi readily grow in it.

As a rule tissues stain better with carmine when they are left in a dilute solution for 12 to 24 hours.

The following method (Heidenhain) frequently gives good results.

The sections to be stained are placed in a watch-glass under a bell-jar, with very dilute carmine prepared as in (*b*). Under the bell-jar there is also placed a dilute solution of ammonium carbonate. The staining is complete in about twenty-four hours.

Picrocarminate of Ammonia or Picrocarmine.

Prepare a saturated solution of picric acid, and to it add a saturated ammoniacal solution of carmine till a precipitate occurs. Evaporate in a water-bath till the volume is reduced by four-fifths. Filter, and evaporate the filtrate to dryness. A crystalline mass of picrocarmine is thus ob-

2. Make a saturated solution of alum in 70 per cent. alcohol
    by the method above given for calcium chloride.   Add this
    to the filtrate in 1 in the proportion of 8 to 1.

3. To the mixture thus obtained, add drop by drop a saturated
    solution of hæmatoxylin in absolute alcohol, till it is a
    moderately dark purple.   In dissolving the hæmatoxylin
    in absolute alcohol remember that it is very soluble, and
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    too deeply, the colour may be extracted by placing the
    section in dilute acetic acid.   At the same time it must be
    remembered that the acetic acid will exert its own proper
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    in a separate bottle, since after filtration it may be used
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a.   Carmine   0·3 grm.
                    Glycerine   30·0   „
              .   Alcohol   4·0   „
                    Distilled water   30·0   „
                    Ammonia   q. s.

Add the distilled water to the carmine, and then add dilute
    ammonia drop by drop until the carmine is dissolved,

taking care that the least possible excess is added. If the solution smells strongly of ammonia expose it in a flat vessel to the sunlight for a day or two. Finally add the glycerine and alcohol, and shake. It should be kept in a stoppered bottle, since otherwise the ammonia evaporates and the carmine is precipitated.

It may be diluted with water to any extent required.

For staining fresh tissue the carmine may be prepared thus:

b. Mix in a mortar 1 grm. of carmine with a little water into a thin paste; add ammonia, drop by drop, till the whole is lake-red, then add 100 cc. of water, and evaporate on a water-bath till the excess of ammonia is evaporated off, and the carmine begins to be precipitated: filter. The excess of ammonia must be got rid of, otherwise the staining becomes diffuse.

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Prepare a saturated solution of picric acid, and to it add a saturated ammoniacal solution of carmine till a precipitate occurs. Evaporate in a water-bath till the volume is reduced by four-fifths. Filter, and evaporate the filtrate to dryness. A crystalline mass of picrocarmine is thus ob-

tained. It dissolves readily in distilled water; a 1 to 5 p. c. solution should be made, and further diluted as occasion requires.

A great deal of the yellow colour may be removed from tissues stained with picrocarmine by careful washing with water.

Aniline blue-black.

Dissolve 5 grm. of aniline blue black in 100 cc. of water. This may be diluted with water to any strength required.

It may also be dissolved in 90 p. c. spirit, to stain tissues that have been placed in spirit.

Gold Chloride.

The method of using this is for ordinary cases sufficiently given in the text (Lesson v. B, § 4).

The following rather complicated method is used by Gerlach to trace fine nerve-fibres in the spinal cord.

The fresh spinal cord is placed from fifteen to twenty days in a 1 to 2 per cent. solution of ammonium bichromate at a low temperature.

Sections are cut and placed in a ·01 per cent. solution of the double chloride of potassium and gold *very* faintly acidulated with hydrochloric acid for ten to twelve hours in the dark. They should be then of a pale lilac colour.

They are washed in hydrochloric acid ·05 per cent., and then left for ten minutes in a mixture of one part of hydrochloric acid to one thousand of sixty per cent. alcohol, and finally placed for a few minutes in absolute alcohol, cleared and mounted in Canada balsam. The nerve-fibres are most distinct in three or four days.

Silver Nitrate.

This is used mainly to trace out flat tesselated epithelium, such as that of arteries, veins, or lymphatics. By exposure to light, the silver is reduced in very fine granules; in a

fresh tissue that has been placed in nitrate of silver the reduction on exposure takes place more readily in the homogeneous substance between the cells or 'cement substance' than in the cells.

As a consequence, where there is a single layer of cells with a small amount of cement between them, the reduced silver in this substance marks very distinctly the outline of the cells.

The success of silver preparations largely depends upon not dragging the tissue, and on washing it well before it is exposed to the light.

The reduction of silver goes on slowly in the other parts of the tissue, so that it becomes darker and darker, until it is useless; this is rendered less rapid by mounting.

MOUNTING.

Some fresh and nearly all hardened tissues are too opaque even in thin sections to enable a satisfactory microscopic examination of them to be made. They are accordingly placed in some fluid which will thoroughly penetrate them and render them more transparent.

Glycerine.

This causes fresh tissues to shrink considerably, since by exosmosis the water is drawn out of them; hence they should never be mounted in concentrated glycerine; if, however, they are not sufficiently transparent they may be mounted in it when diluted with two parts of water. Even with hardened tissues it is often better to use the dilute fluid. It must be remembered that sections which have been in alcohol should be placed in water a short time before mounting in glycerine, otherwise, as alcohol and glycerine do not readily mix, small bubbles of alcohol will make their way to the surface and spoil the specimen.

Glycerine, besides clearing the tissue, acts as a preservative agent; specimens mounted in it may be kept for a con-

siderable length of time. When it is desired so to preserve a specimen, care should be taken that the drop of glycerine is of such a size that the simple pressure of the cover-glass will cause it to spread out just to the edges of the cover-slip ; the glass slide round the cover-slip and the edges of the cover-slip should be overlaid with tolerably consistent Canada balsam. If too large a drop of glycerine is taken, so that it spreads out past the edges of the cover-slip, the Canada balsam will not stick to the glass. In this case it is generally less trouble to remount the section than to wipe away the glycerine.

Clearing with Carbolic Acid and Turpentine.

Add 1 part of carbolic acid to 4 of turpentine. The carbolic acid may be replaced by creosote; in this case shake well and set aside till the cloudiness, which forms on mixing the liquids, disappears. This mixture is used for stained and injected sections which it is desired to render very transparent; they must be mounted in Canada balsam. The carbolic acid (or creosote) is the clearing agent, but this alone cannot be used since it does not dissolve Canada balsam, accordingly turpentine is added, which mixes with the acid and also dissolves the balsam. Gently warming in carbolic acid and turpentine hastens the clearing.

Before mounting sections all superfluous fluid should be removed by means of a piece of blotting paper with the edge not torn but cut clean.

Injected specimens should, as a rule, be cleared with carbolic acid and turpentine; if soluble Berlin blue has been used as the injection material, it is advisable to leave the sections in a little turpentine in an uncovered watch-glass for a day to restore the colour which probably has been weakened by alcohol.

Canada Balsam.

Put some Canada balsam into a capsule and place it in the warm chamber at about 65°C. for twenty-four hours to drive off all water. Let it cool and dissolve it in a small

quantity of benzole; it should be kept in a bottle with a ground glass cap fitting over the neck of the bottle, instead of a stopper, as the stopper is apt to become fixed in the bottle; the neck of the bottle should be rubbed round with lard, to prevent the cap sticking. Dammar may be used instead of Canada balsam.

Canada balsam renders tissues much more transparent than glycerine.

Tissues stained in carmine or any watery solution should be placed in absolute alcohol previously to being mounted in Canada balsam. It is however most used for tissues which have been cleared with carbolic acid and turpentine.

Acetate of Potash.

A saturated solution of this is often used to mount osmic acid specimens in.

GENERAL DIRECTIONS.

Fresh specimens should be teased out or examined in normal solutions.

Osmic acid specimens should be teased out in the fluid itself or in water.

Potassium bichromate and ammonium bichromate specimens should be teased out in the fluid itself or in water; dilute glycerine may also be used, but is commonly not necessary.

Chromic acid specimens should be teased out in water or dilute glycerine.

Sections hardened in potassium bichromate, chromic acid, &c. when unstained should be mounted in glycerine.

When stained they should be mounted in glycerine or cleared and mounted in Canada balsam.

Gold chloride specimens should be mounted in glycerine and kept in the dark.

Silver nitrate specimens should be cleared and mounted in Canada balsam.

15—2

Never mount in glycerine sections cleared with creosote and turpentine.

Millon's reagent.

Weigh out 50 grms. of purified mercury and an equal weight of pure strong nitric acid. Place the mercury in a flask in the closed chamber, add the nitric acid to it, and leave the mixture as long as any chemical action continues. If all the mercury is not dissolved warm it *gently* to complete the solution.

Add then to it twice its volume of water, and place aside for some hours; a white crystalline precipitate will fall; the supernatant fluid is Millon's reagent.

Fehling's fluid.

(*a*) Dissolve 34·65 grm. of pure crystallized cupric sulphate in about 160 cc. of distilled water.

(*b*) Dissolve also 173 grm. of pure crystallised potassic-sodic tartrate in 600 to 700 grm. of sodic hydrate sp. gr. 1·12.

Add (*a*) to (*b*) stirring well to cause a thorough mixture, and dilute with distilled water to a litre.

Fehling's fluid should be fresh made whenever it is required, since it decomposes on keeping; it will keep some little time if kept in a cool place in the dark and in completely filled well closed bottles (Hoppe-Seyler).

The solution (*b*) may be prepared and kept for adding to (*a*) freshly prepared when required.

Before using a kept solution to test for sugar always boil a little of it by itself to see if any reduction will take place.

From 1 cc. of this solution the copper is completely reduced by ·005 grm. of grape-sugar.

Stokes' fluid.

This should be made afresh when required; it consists of
a solution of ferrous sulphate, to which ammonia has been
added after the previous addition of sufficient tartaric acid
to prevent precipitation. An excess of tartaric acid is
not of consequence; roughly speaking, three parts by weight
may be added to two parts of ferrous sulphate.

MAJOR ARTERIAL SCHEME.

This (see Fig.) is a modification of Weber's original scheme of
the circulation. It is not intended to imitate the details
of the circulation of the blood, but simply to illustrate
the points to which attention is called in Lesson XII.
It can without much difficulty be made by joining to-
gether india-rubber tubing by means of three-way glass
tubes.

Clamps are placed upon the straight tubes C, C', C'', and
the dilated tubes along the same line are stuffed with

sponge, until on closing the clamps and pumping steadily, a mean pressure of two or three inches of mercury is obtained in the artery tube.

The artery tube *P* is connected with an egg-shaped india-rubber bag; from the opposite end of which a tube passes to a vessel full of water. The bag has two valves, one at each end, opening in the same direction, so that when it is compressed with the hand the water it contains is driven onwards through the artery tube, and on its dilating water is drawn up from the vessel.

Any other form of pump can be used.

At *S, S, S,* are placed vertically above one another either the usual sphygmograph levers or light levers such as are used to take tracings of the movements of the frog's heart (Lesson XI. B). The tubing can be supported on pieces of lead fastened to a stand, and bent to fit its course. Each of the tubes *A* and *V* communicates with a mercurial manometer (Hdb. p. 210).

The rest of the scheme requires no further explanation than is given in the text.

Minor Arterial Scheme.

This consists of an india-rubber bag like that used in the major arterial scheme. By means of a three-way tube the end of this towards which the valves open is connected both with a piece of glass tubing about a yard and a half in length, and with a piece of india-rubber tubing of similar length and bore.

There are clamps upon the long india-rubber tube close to its junction with the three-way tube and upon the small piece of india-rubber which connects the three-way tube with the glass tube, so that the flow of water may be through either the glass or the india-rubber tube.

A small piece of india-rubber tubing is also placed on the end of the glass tube, into which a tube finely drawn out can be inserted.

PITHING.

If it is desired to pith a frog, place it under a small bell-jar with a sponge moistened with ether; when the animal is motionless and under the influence of the anæsthetic, take it up in a cloth, hold the hind legs between the third and fourth fingers, and with the fore-finger press down the head. Cut transversely through the skin a little behind the skull with a pair of scissors, carrying the incision only a short way on either side of the median line; make a slight incision also through the skin in the median line. The end of the occipital bone can then be easily made out; it is just above the level of the anterior edges of the scapulæ. With a sharp scalpel cut through the muscles just behind the occipital bone and as nearly as possible in the median line, so as not to cut the vertebral arteries; the spinal cord will be exposed. Thrust then a blunt stout needle into the brain and move it about so that the brain is quite destroyed.

The following rough table may be useful to the student.

1 litre	= 1 cubic decimetre = $1\frac{3}{5}$ pints.
1 cubic inch	= 16·39 millilitres or cubic centimetres.
1 cubic foot	= 28·32 litres.
1 gallon	= 4·54 litres.
1 gram	= 15·43 grains.
1 kilogram	= 32·15 Troy oz. = 35·27 Avoird. oz.
1 grain	= 0·065 gram.
1 Troy ounce	= 31·1 gram.
1 pound (Avoird.)	= 0·45 kilogram.
1 centimetre	= $\frac{2}{5}$ inch.
1 metre	= 1 yard $3\frac{1}{3}$ inches.
1 inch	= 2·54 centimetres.
1 foot	= 3·05 decimetres.
1 yard	= 0·91 metre.

INDEX.