Manual for the physiological laboratory / by Vincent Harris and D'Arcy Power.

MANUAL

FOR THE

PHYSIOLOGICAL LABORATORY.

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BY

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PREFACE TO THE FIFTH EDITION.

It has been found necessary to increase the size of the fifth edition of this work. This increase is mainly due to the more detailed account which has been given of microscopes, and of their properties for junior students; whilst for senior students special care has been taken to describe the latest methods of Histological Research. The Physiological Chemistry has been thoroughly revised, and has been made more complete than in the previous editions: at the same time the experimental exercises in Part III. have been adapted to the requirements of students who are preparing for the higher examinations in Physiology.

Many additional illustrations have been inserted. For the use of some of the new figures we are indebted to the courtesy of the Cambridge Scientific Instrument Company, Herr Zeiss of Jena, Messrs. Arnold, Messrs. Elliott Bros., Mr. Thistleton, and Mr. John Murray, who has kindly placed at our disposal figures 76, 77, 78, 100, 101, 105, 111, taken from the last edition of Kirkes' 'Handbook of Physiology.'

November, 1891.

PART I.

PRACTICAL HISTOLOGY.

CHAPTER I.

THE MICROSCOPE AND ITS ACCESSORY APPARATUS.

The study of Practical Histology may be conveniently introduced by an account, first of all, of the microscope and its accessory apparatus, and next of the methods of preparing tissues by means of which their minute structure may be adequately demonstrated. It will be as well, however, to consider the two parts of the subject separately, and to devote the whole of the present chapter to the first part, viz., to a description of the microscope and its uses, and to give up the following chapter to an account of the methods of histological research.

The microscope which may be most advantageously selected for description is the kind known as the **Continental model**. This form was originally manufactured by foreign makers only, and differed both in size and plan from the microscopes made in this country. For some years, however, instruments very similar in construction have been introduced by most of the best English makers. It should be remembered that this variety of instrument is often called the **Hartnack model**, as it was chiefly by means of Hartnack's microscopes that the smaller, as opposed to the larger, form of microscope became popular among histologists.

The **stand** or body of the instrument (Fig. 1) consists of two parts. Of a base of solid brass, into which is fixed vertically a substantial brass pillar about two inches high, to which is attached by a hinge the second portion, made up of a hollow brass cylinder, carrying below at right angles the **stage** of the microscope, and above, by a projecting arm, a long brass collar, in which the **tube** of the microscope may be made to slide. At the top of the hollow cylinder is a screw with a milled head which works the **fine adjustment**, by means of which the tube of the microscope may be moved very slightly to or from the stage. In the simpler forms the **coarse adjustment**, or the larger movement of the microscope tube up and down, is effected by the hand, the fingers of which,

grasping the tube firmly, rotate it downwards or upwards, according as it is necessary to bring the tube nearer to or to carry it further away from the stage; in the more expensive models however these larger movements are performed by a rack-and-pinion apparatus (Fig. 14). The **stage** of the microscope, upon which the object-glass is placed, is a flat plate of brass blackened upon its upper surface, or of glass cemented on to a dark background, and perforated by a central aperture. Through the aperture light is reflected from below by a **mirror**; the amount of light admitted to the lens is regulated by a

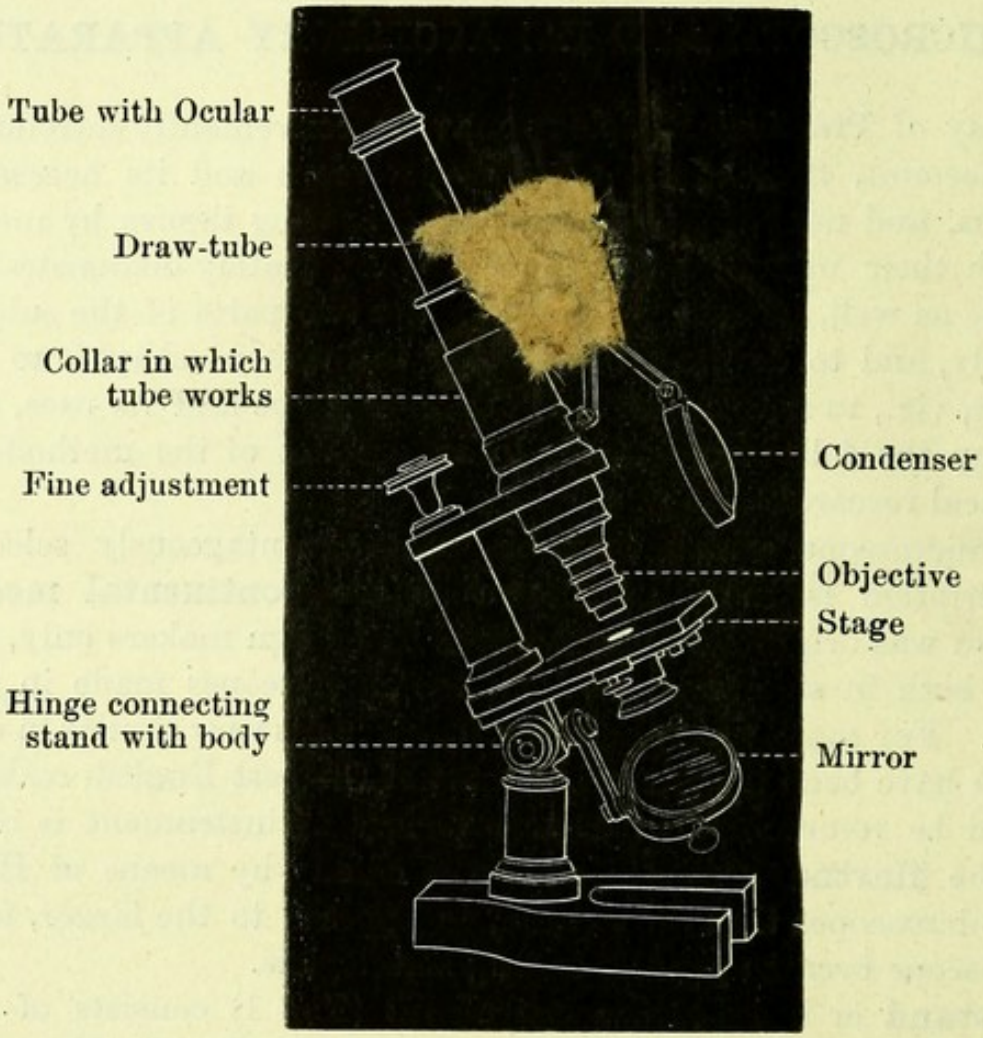

Fig. 1.—The Continental-Model Microscope.

small cylinder which fits into the central aperture; this, when withdrawn from below, will receive the **diaphragms**; these are perforated by apertures of different sizes; the smallest is a mere pinhole, and is for use with the strongest magnifying power. One of the diaphragms should be inserted into the cylinder, and replaced in the centre of the stage. When in position it is exactly flush with the upper surface. In some microscopes this form of diaphragm is replaced by a blackened disc of metal perforated near its circumference by

holes of various sizes, and revolving round its centre. In the newer microscopes the so-called **Iris** diaphragm (Fig. 2) is used, in which, by moving a small handle, the size of the aperture may be varied at pleasure. At the back of the stage is a pair of brass **clips** for holding the slides in position when the microscope is tilted. In some instruments the movements of the stage in different directions are effected by screws; but in this the stage is fixed, and the finger and thumb suffice to move the slide upon it.

Beneath the stage is a movable **mirror** with two faces, one concave, the other plane; the concave mirror is the more commonly used, as it condenses the light upon the object, and thus affords a better illumination. The **tube** of the microscope consists of a hollow brass cylinder from five to eight and a half inches in length. It contains a second or **draw** tube, which can, if necessary, be drawn

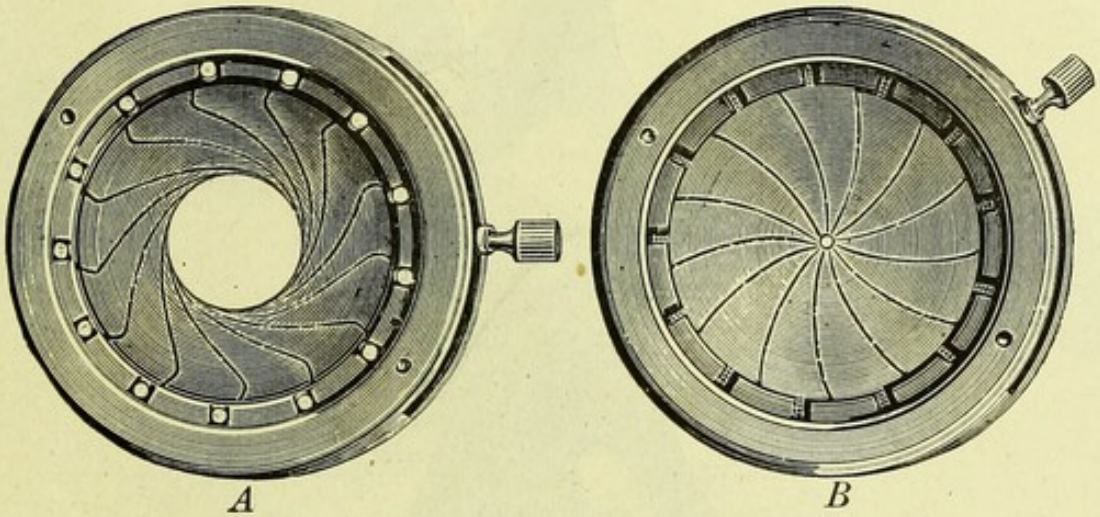

Fig. 2.—Iris Diaphragm. *A*, half open; *B*, closed.

out, in order to increase the magnifying power. The upper extremity of the tube receives the **ocular** or eye-piece: the oculars vary in magnifying power.

Into the lower end of the tube the **objectives, powers**, or **lenses** are screwed. These also vary in magnifying power.

In using the microscope, the low power should be put on first; then the mirror should be adjusted in such a way as to get a full illumination of **the field**, so that the eye applied to the ocular sees a circle of light of equal intensity in all its parts. The direct rays of the sun should not be used. The object to be examined should be placed upon the stage in such a way as to be over the centre of the aperture, the largest aperture of the diaphragm being used. The lens should be brought to within half an inch of the slide

with the coarse adjustment; then the fine adjustment should be carefully employed (focussing), until the object is distinctly seen, the focus being altered so as to observe it at various depths. After examining with the lower power, this should be removed and replaced by higher powers in turn; at the same time the aperture of the diaphragm should be reduced. The lens should be brought down until it almost touches the object, and then by means of the fine adjustment should be focussed upwards.

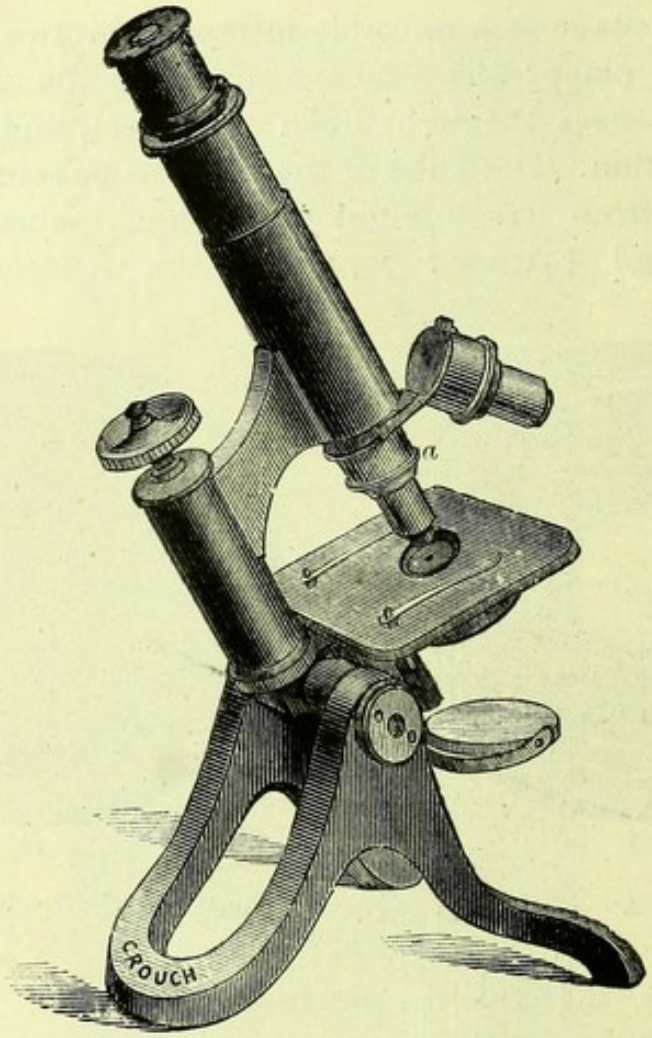

Fig. 3.—English-Model of Student's Microscope.—The nose-piece (*a*) carries two objectives.

Students are apt to leave a portion of one lens attached to the tube when they change it for another; they are also prone to produce obscurity of the field of the microscope by allowing drops of glycerine, Canada balsam, etc., to adhere to the surface of the lens. Attention should be directed to both of these points when it is found that the object under examination cannot be focussed clearly. A drop of methylated spirit upon a soft handkerchief is sufficient, with gentle rubbing, to remove Canada balsam from lenses. A **condenser**, or convex lens, fitted into a jointed lever which slides up

and down the tube of the microscope, is provided; it is employed for condensing light upon opaque specimens; it may, however, be removed, since this form of condenser is scarcely ever employed in modern histology, the majority of specimens being prepared for examination by reflected light.

It will be seen by an examination of Fig. 3, which represents a student's microscope of English make, that this differs but little from the model we have described. The body or stand is, however, a tripod arrangement, which is the form chiefly in vogue at the present time.

Before giving a brief description of the optical plan of a compound microscope, into the construction of which many lenses enter, we may mention, in passing, **the simple or dissecting**

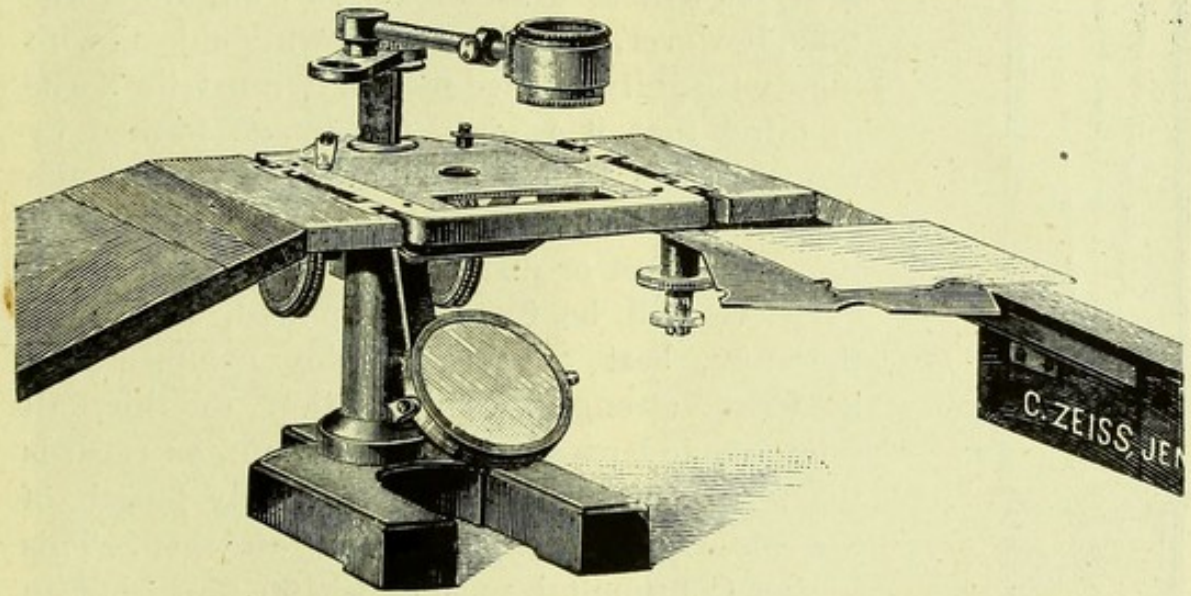

Fig. 4.—Dissecting Microscope (Zeiss).

microscope, which consists essentially of a stage illuminated from below by a movable mirror. Above the stage is an arm which can be raised or lowered by a rack and pinion. The arm carries a lens similar to that employed by watchmakers. The dissecting microscope is serviceable in certain cases where it is necessary to tease or otherwise manipulate very delicate tissues. It is, however, chiefly employed by biologists.

The Mode of Action of the Compound Microscope.—The rays of light (Fig. 5) which proceed from the object under examination are focussed behind the objective at a point in the lumen of the tube of the microscope the position of which is dependent upon the strength of the magnifying power employed. Thus, the higher the objective, the nearer is the object focussed to the lower end of the tube; and the less the magnifying

power of the objective, the higher in the tube is the focus. After the various rays have been brought to a focus they cross each other and become divergent, until they are a second time gathered up by the front lens of the ocular, which focusses them within the eye-piece at the level of the diaphragm. An enlarged but inverted image of the object in front of the objective is thus formed, which is a second time magnified by the lens of the ocular nearest to the eye. The magnifying power of the microscope is increased within certain limits by increasing the distance between the objective and the eye-piece, and it is for this reason that most of the modern instruments are provided with a 'draw-tube.' In using the draw-tube, however, as well as in viewing objects with highly magnifying eye-pieces, it must be borne in mind that it is only the image formed by the objective which is enlarged, and not the object itself, and with such increased magnification any defect or distortion on the part of the objective will be further amplified, and it is, therefore, best only to employ eye-pieces of moderate strength. In addition to this, all microscopic lenses have an optimum, as regards the length of tube, or so-called body length, of the microscope to which they are attached. This in the Continental models is 160 mm., but in English instruments may be 250 mm. At any other distance the lenses do not perform so well.

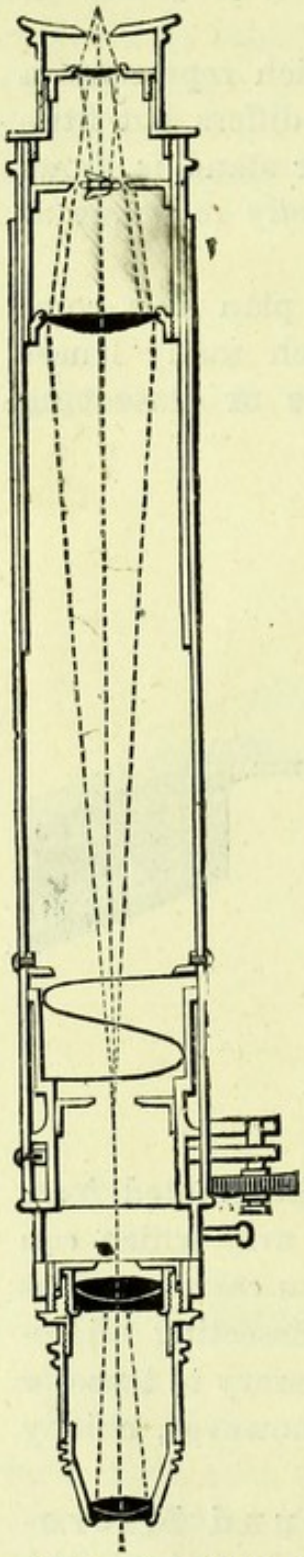

Fig. 5. — Optical plan of a Compound Microscope.

The Construction of Objectives. —In Fig. 5 the objective, although one of low magnifying power, is seen to be made up of combinations of lenses instead of single plano-convex glasses. This is the plan always adopted nowadays with the compound microscope. Objectives of high magnifying power, indeed, are always made up of more than two such combinations. The explanation is not far to seek.

Single plano-convex lenses are subject to two great defects, of which the first is **spherical aberration**. This results from the fact that rays of light which pass through the circumference of the lens

are brought to a focus at a point nearer to the lens than the rays which pass through the centre. The second defect is that of **chromatic aberration**, consequent upon the complex nature of white light. The rays in passing through the lens are split up into their component parts, and thus the colours of the spectrum are obtained in the same way as when light passes through a prism. Each colour of the spectrum is focussed at a different point, and the image is therefore surrounded by a coloured fringe. It has been found, however, that by a skilful combination of different kinds of glass, objectives can be manufactured in which the spherical and chromatic aberrations are practically done away with. Objectives, therefore, are usually composed of combinations of lenses, varying in number from two to eight, such as we have above mentioned,

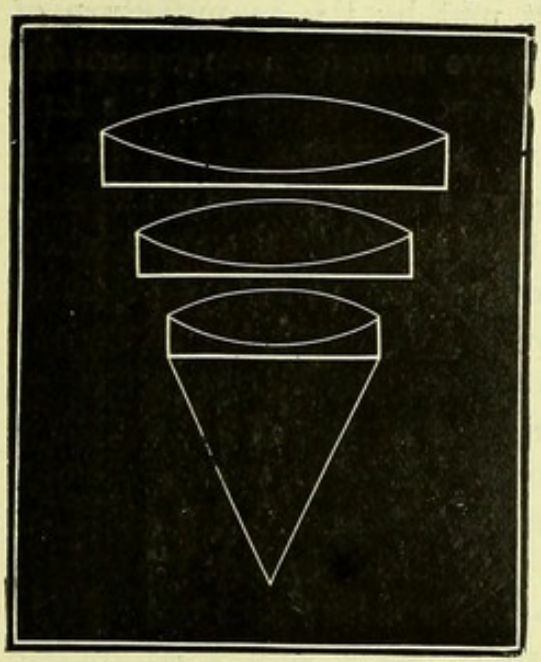

Fig. 6.—Old Form of Combination of Lenses.

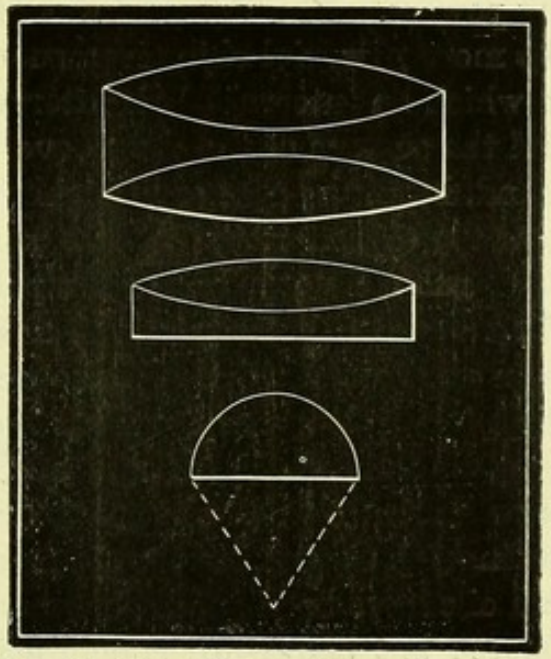

Fig. 7.—New Form of Combination of Lenses.

and they are composed of flint and crown glass; the flint-glass lenses being concave, and ground so as to fit the opposed convex surfaces of each crown-glass lens. In the better class of objectives the lenses farthest away from the object are ground in such a manner as not only to correct their own spherical aberration, but also that of the front lens. By this means an objective is obtained in which the front lens consists of a single glass, and not, as in the older forms, of an achromatic combination of convex and concave glasses, which only allow of a small angular aperture.

By the **angular aperture** of a lens is meant the number of rays of light which it is capable of transmitting. Such aperture is measured by observing, with a special instrument, the angle subtended from the centre of the objective by the most oblique ray of

light transmitted on either side. For ordinary purposes an objective whose focal length is $\frac{1}{4}$ inch should not possess a greater angular aperture than 100°; whilst $\frac{1}{8}$-inch should not exceed 130°, as beyond this point the larger amount of light entering the objective is more than counterbalanced by the increased proximity of the lens to the object, and the diminished amount of penetrating power which such a glass possesses. This statement does not hold true for immersion lenses, in which the angular aperture can be increased to a very great extent without detriment to the utility of the objective.

Immersion Lenses.—The development of modern histology, especially in the direction of the recognition and description of the exceedingly minute forms of vegetable life known as bacteria, has led to a corresponding demand for higher microscopic magnifying powers. This demand was at first met by the manufacture of the more powerful objectives such as have already been described, in which an interval of air intervenes between the front of the lens and the surface of the glass covering the preparation. It was soon found, however, that such higher objectives focussed inconveniently near the object, whilst at the same time they had a very limited field, and did not admit of the passage of sufficient rays of light to yield a good illumination. A new form of objective, therefore, has been constructed of such a character that it focusses an object when the interval between the front lens and the cover-glass is occupied by some fluid which reduces to a minimum differences in the index of refraction. Such objectives are known as 'immersion' lenses, to distinguish them from the old form of 'dry' lens. Water, glycerine, and oils have been successively employed in connection with these lenses; but **cedar-oil**, condensed until it has a somewhat higher specific gravity than usual, is now most commonly used for the purpose, because its index of refraction is found to be very nearly equivalent to that of crown-glass, and, therefore, nearly all the rays of light which are focussed upon the object pass through the objective into the body of the microscope.

To use an immersion lens, the mounted specimen must be sealed with marine glue, gold-size, or other cement which is not soluble in cedar-oil. A small drop of the clear oil should then be lightly placed, by means of a clean glass rod, upon that portion of the cover-glass which is immediately over the part of the preparation to be examined. The lens should afterwards be brought down carefully until its surface is in actual contact with the oil, and should then be focussed by means of the fine adjustment. The immersion lenses employed in histological research are usually without correction for the thickness of the cover-glass, as they are more readily employed than when a

different correction of the lens has to be made for each thickness of cover-glass employed. Before using the immersion lens it will usually be found expedient to find the particular spot in the preparation with an ordinary high power, and if the specimen is permanent the part required should be marked out for future reference by drawing round it upon the cover-glass a small ring of cement or ink.

In the majority of cases a special form of microscope, such as is described at p. 17, will be required, as it will be necessary, in order to obtain a sufficiently powerful illumination, to attach an achromatic condenser beneath the stage. After using the immersion lens, it should be at once unscrewed from the microscope, its front surface should be gently wiped with a soft handkerchief or piece of chamois leather kept for the purpose, and it should then be replaced in its box.

Apochromatic Objectives.—A new series of lenses, termed Apochromatic, has been introduced by Zeiss. The particular advantages claimed for them are that in consequence of an improved method of correction, and the use of new kinds of glass, the spherical aberration is entirely abolished. The lenses can be employed with much higher eye-pieces than has hitherto been the case; and the natural colour of objects, even in the more delicate tints, are reproduced unaltered. To obtain the best results with these objectives, they should be employed with the **compensating eye-pieces** manufactured by the same firm; when this is done an image is obtained which is uniformly free from colour throughout the whole field of view. It seems that at the periphery of the field, even when apochromatic objectives are used, certain colour defects are perceptible, the blue image of the object being greater than the red. The eye-pieces are so made that they magnify the red more than the blue, and the result is that they compensate the different magnification of the objective, and even at the edge of the field the images are free from colour.

In an interesting article on Abbe's apochromatic micro-objectives, Mr. Schulze makes the following observations upon microscopic lenses, which may be looked upon as, to a certain extent, an epitome of what has been already said: 'A good micro-objective is a system of lenses, often consisting of as many as twelve, which are composed of crown and flint glass ground to certain spherical curves. In selecting an objective attention should be paid to the following details: First of all, its defining power or **definition**, which depends upon the more or less perfect correction of the spherical and chromatic aberration. Next comes the **resolving power**, or the power to separate and make visible close structural details, surface-markings, bands of lines, and gratings. This power is dependent upon the

aperture of the lens, which is the power to collect and transmit a smaller or larger cone of rays from the object to the eye-piece. Then comes the **penetrating power** or focal depth, which consists of the vertical range, through which parts of an object of different planes can be seen at the same time. Narrow-angled lenses, however, possess greater penetrating power than those with wide angles of aperture. The **flatness** is that quality of a lens which permits of an image being seen with the same distinctness in the centre as at the margin of the field without any readjustment of the focus. The **working distance** has no fixed relation to the focal length, but wide-angled lenses have shorter working distances than lenses of narrow angles. A good objective should combine all the foregoing qualities; but if for special purposes some of them have to be sacrificed, first-rate definition should be preferred to every other quality. Next in importance to the definition of a lens is its resolving power, because the aim in microscopy is not to magnify as many times as possible, but to see microscopical details clearly with as little magnification as practicable, mere magnification being easily obtainable by putting on a high eye-piece, which enlarges all the details brought out by the lens.'

Oculars or eye-pieces are of two great types. The first, or Huyghenian, is the form in common use, and consists of two plano-convex lenses, separated by a distance equal to half the sum of their combined focal length, with a diaphragm placed between them. The second, or Kellner, or orthoscopic eye-piece, is chiefly employed in photo-micrography, and is composed of an upper achromatic lens, and of a lower bi-convex lens placed in the focus of the upper or eye lens. It has no intervening diaphragm, and, therefore, has nearly double the field of view possessed by the Huyghenian eye-pieces.

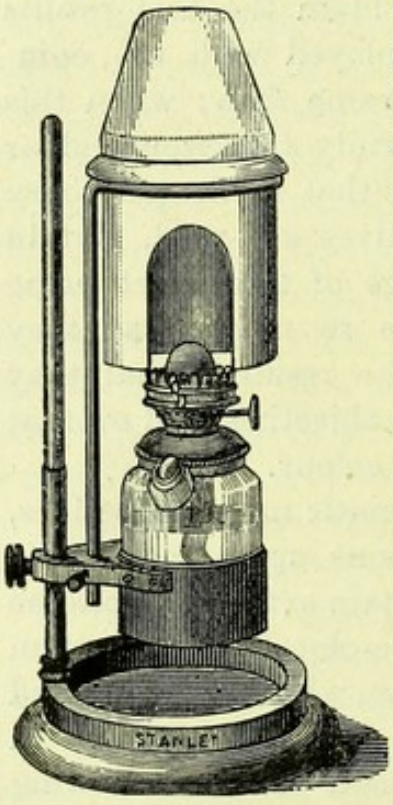

Fig. 8.—Microscope Lamp.

Light.—The best light for microscopic work is that afforded by the sun, when its rays are not too powerful. A fine sunny spring day affords what may be called a typically favourable light. The light should be considered best when reflected from white clouds in a blue sky. In England, however, only too frequently artificial light has to be made use of. Various forms of gas and oil lamps have been suggested. A drawing (*e.g.*, Fig. 8) of a convenient form is given. As a rule we employ gas argand burners, with blue glass funnels. The light is generally

transmitted to the object by a slightly concave mirror through a diaphragm. The illumination of objects when opaque is done, as before mentioned, by means of condensers of various kinds, which focus the light upon the object from above, or condense it laterally by parabolic side-reflectors. Opaque objects, however, seldom need to be examined in histological work.

In using immersion objectives, the ordinary mirror is supplemented by some form of achromatic condenser, of which perhaps the illuminating apparatus designed by Abbe may be considered as the best. It consists essentially of a system of two or more lenses, of short focus, so combined as to transmit a large pencil of achro-

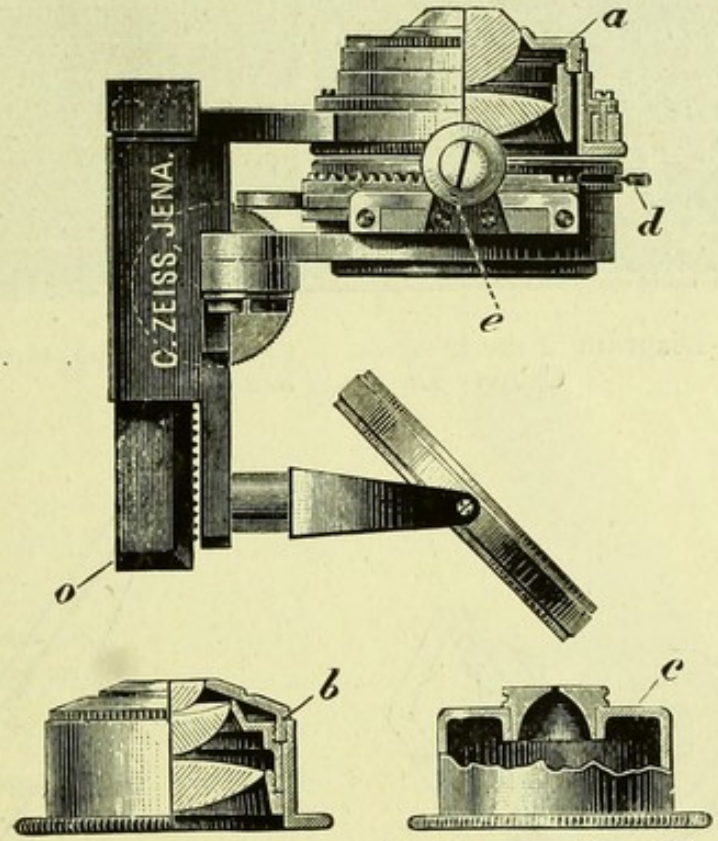

Fig. 9.—Apochromatic Condenser, after Abbe.

a, condenser system of 1·2 numerical aperture; *d*, iris diaphragm; *e*, adjustment for throwing the diaphragm out of centre; *c*, cylinder diaphragm; *b*, condenser system of 1·4 numerical aperture; *o*, bar along which the apparatus works.

matic light, which is reflected either from a concave or plane mirror. The amount of light entering the instrument may be regulated by introducing diaphragms; whilst the direction of the rays can be varied at will by turning a milled head. The top lens, when the apparatus is in position, fits accurately into the central aperture in the stage of the microscope, and is flush with its upper surface.

Of Drawing Microscopical Objects.—If the student is a good draughtsman, practice is the only thing required before he can make good drawings of microscopic objects. In others practice

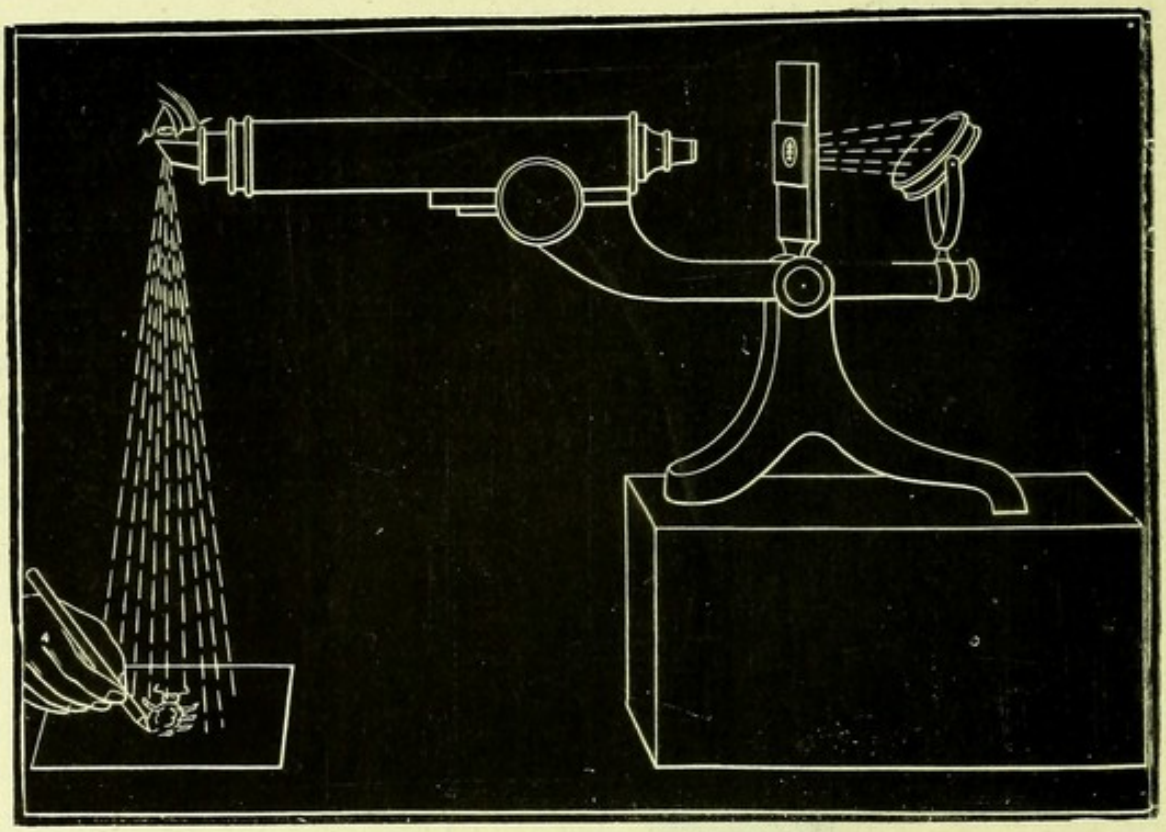

Fig. 10.—Diagram of the Position of the Microscope, etc., when the Camera Lucida is being used.

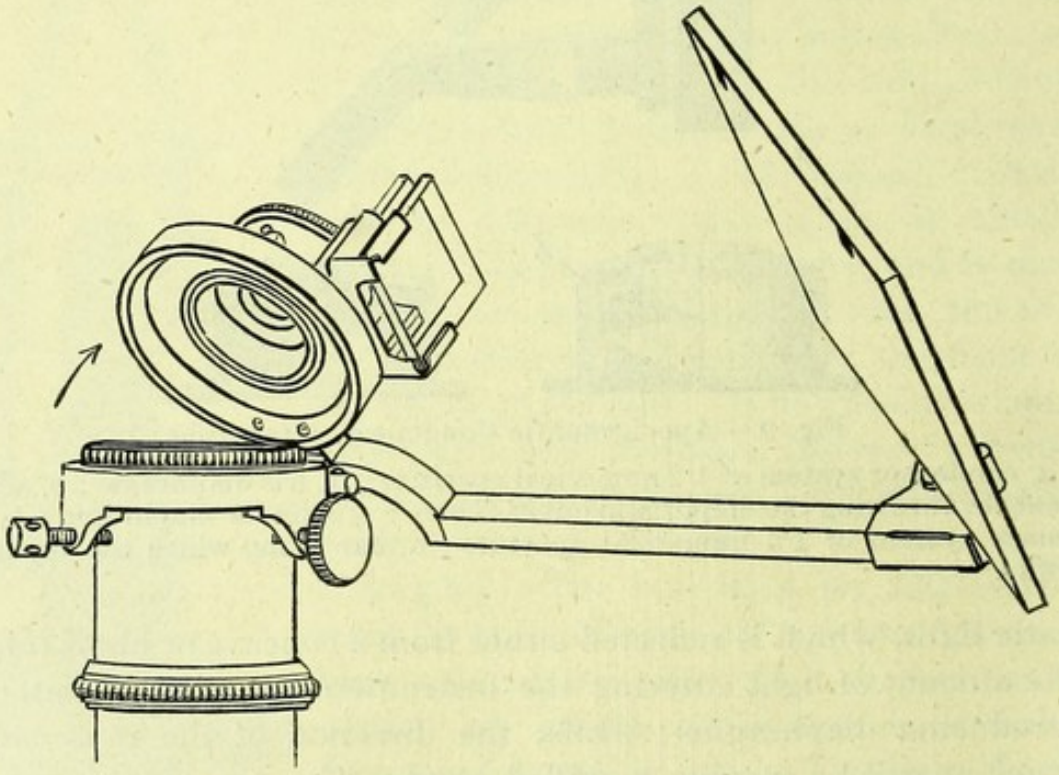

Fig. 11.—Camera Lucida, after Abbe.

In this camera lucida the drawing surface is made visible by a double reflection, from a large plane mirror and from the silvered surface of a small prism in the eye-point of the eye-piece. The microscopic image is seen directly through an aperture in the silvering of the prism. In this way the pencil of rays reaching the eye from both the microscope and the paper, the image and pencil are seen coincidently without any straining of eyes. The brightness of the paper is regulated by smoke-tinted glasses which fit into the prism casing.

will very possibly bring out latent talent. Some, however, whatever their perseverance, will need aid; and this is given them by an instrument called the **camera lucida**. This consists of a prism arranged in such a manner that, when attached to the microscope-tube by a holder, and placed exactly over the eye-piece, the image of the object is thrown upon some part of the table where a sheet of white paper may be laid, and the outline of the object can then be traced upon it. An instrument constructed for a similar purpose is Beale's neutral-tint reflector, which is thus used: The cap of the eye-piece is removed, and the reflector is applied in place of it. The microscope should then be inclined to a horizontal position, and at ten inches from the table, and the paper should be placed exactly underneath the reflector. After the object is focussed and properly illuminated, the eye should be brought *close to* and *exactly over* the reflector. The image will then appear to be thrown upon the paper, and may readily be traced.

DIRECTIONS FOR CHOOSING A MICROSCOPE.

As great care is necessary in the choice of a microscope, the student is recommended not to buy one without asking the advice of someone who is well acquainted with modern instruments. The supply of different kinds is now so extensive, that a description of what should be chosen is difficult, and might be invidious. We may, however, tell him roughly *what not to get*. Let him *not* buy one of those large constructions of brass which are so often strongly recommended by dealers, or one which has a complicated arrangement of screws and buttons to move the object-glass. This would be simply paying money for useless material. Binocular microscopes cannot be recommended, or those in which the fine adjustment tilts the draw-tube forward. Second-hand microscopes, except modern instruments of well-known makers, are to be avoided, as are also old

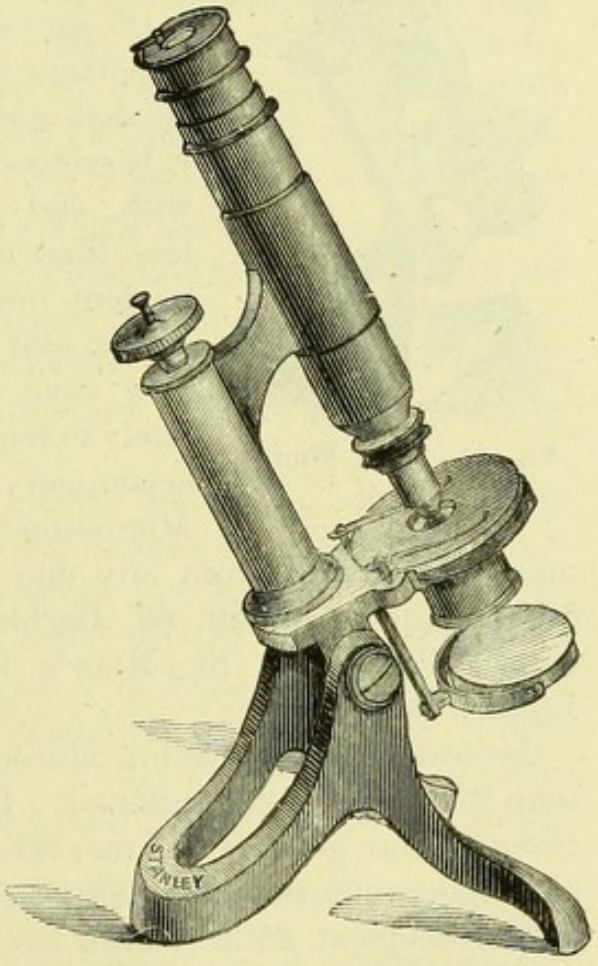

Fig. 12.—Student's Microscope.

instruments, of whatever kind. The following points of advice may be of some use:

The stand must be small, and at the same time firm; the fine adjustment delicate and steadfast. The oculars ought to be clear and achromatic, free from flaws, scratches, and spherical aberration.

The lenses should also be free from any such faults, and should exhibit as flat a field as possible, the whole of the field being in focus at once; should have a fair power of penetration—*i.e.*, should be capable of showing the parts beyond the exact focus; and should also possess what is technically known as resolving power—*i.e.*, should be able to focus clearly a number of fine lines, close together, in an object.

Every part should be carefully tested, and if found defective should be returned to the maker after a fair trial, to be exchanged for a more perfect instrument. The best makers willingly allow this, and indeed are anxious that no imperfect microscope or lenses bearing their names should be in current use.

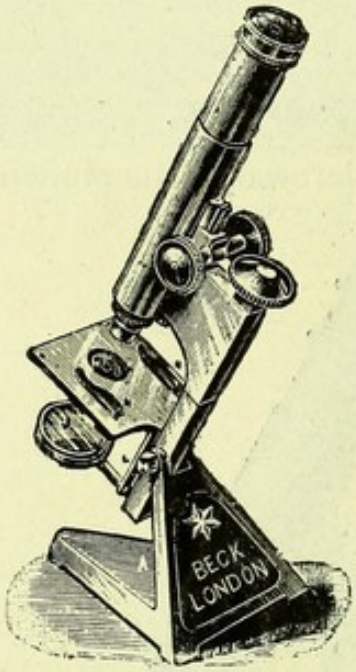

Fig. 13.—Star Microscope.

Of course the question of *price* is a very important one—how much money is to be expended upon the purchase of an instrument? It is seldom necessary to spend more, to begin with, and not expedient as a rule to spend less, than *five guineas*. For five guineas the student may obtain a good stand, two eyepieces, and two objectives, $\frac{2}{3}$-inch and $\frac{1}{5}$ or $\frac{1}{6}$, in a stout case; in fact, all that he will be likely to require for a considerable time. The accompanying figure (Fig. 12) of a Student's Microscope shows such an instrument. It may be obtained from any one of the following makers: Beck, 68, Cornhill; Crouch, 66, Barbican; Swift, 81, Tottenham Court Road; Parkes, 5, St. Mary's Row, Birmingham; or Stanley, London Bridge.

Several makers have introduced microscopes even lower in price than the ones above mentioned. Beck's Star Microscope (Fig. 13), at *three to four* guineas, is a marvel of cheapness. Crouch and Parkes also make microscopes at £3 10s. to £4. Leitz of Wetzlar supplies a microscope at £5 5s. (stand No. IV., with lenses 3, 5, 7, oculars I. and III.), quite suitable for students' use, and one at £3 10s. (stand No. V., lenses 3 and 7, and oculars I. and III.).

Those who wish to purchase an English stand, with German lenses, will find Messrs. Baker's stand, and Zeiss's A and D lenses, a good combination.*

OF THE LARGER MICROSCOPES.

It should be remembered that the student may, in the future, be called upon to do microscopic work which requires the best instruments and lenses, such, for example, as in the study of Bacteria. A question arises, therefore, which perhaps he ought to consider in the initial purchase of a microscope, viz., whether it would not be worth his while either to purchase in the first instance, and once for all, a more complete instrument than the small and cheaper forms just described, or, at any rate, to obtain a more expensive stand capable of being fitted, should occasion arise, with the best lenses, oculars, and sub-stage condenser. It will be as well here to give some information upon this subject. In the first place it should be remarked that all the best English makers manufacture larger and more elaborate instruments than the ones already indicated, and in the next place that many expert microscopists greatly prefer these English-made instruments to any other form, especially the microscopes made by Pillischer, Powell and Leland (whose lenses have a world-wide reputation), and others. Having thus indicated that all do not agree with us, we confess that as far as we ourselves are concerned we greatly prefer the larger microscopes which are made by continental firms, and particularly those made by Zeiss of Jena. This maker has had the great advantage of the assistance upon all optical questions of the high scientific knowledge of Professor Abbe, so that his instruments have been elaborated in accordance with all the newest discoveries in the science of optics.

As regards Zeiss's stands, No. I., with the coarse adjustment by rack-and-pinion movement, the fine adjustment by micrometer screw with divided head, the draw-tube marked into millimeter divisions, the stage fitted with a sub-stage condenser of Abbe's form and capable of adjustment by means of a rack-and-pinion apparatus and iris-diaphragm, but without mechanically movable stage (which we do not recommend), costs £15. A similar form with a short and very wide body, and some other modifications suitable for photo-

* It may appear unfair to mention the names of certain makers, and to omit the names of others. In a practical book, however, it is evident that *definite* advice should be tendered. *We recommend the microscopes the performances of which we have had opportunities of judging.* Other microscopes, of which we have had less experience, may possibly be as good as those recommended.

micrography (Fig. 14), costs £21. Smaller stands, with similar accessories to No. I., but on a somewhat smaller scale, may be had at £14 10s. (Stand II.*a*) and £10 (Stand IV.*a*).

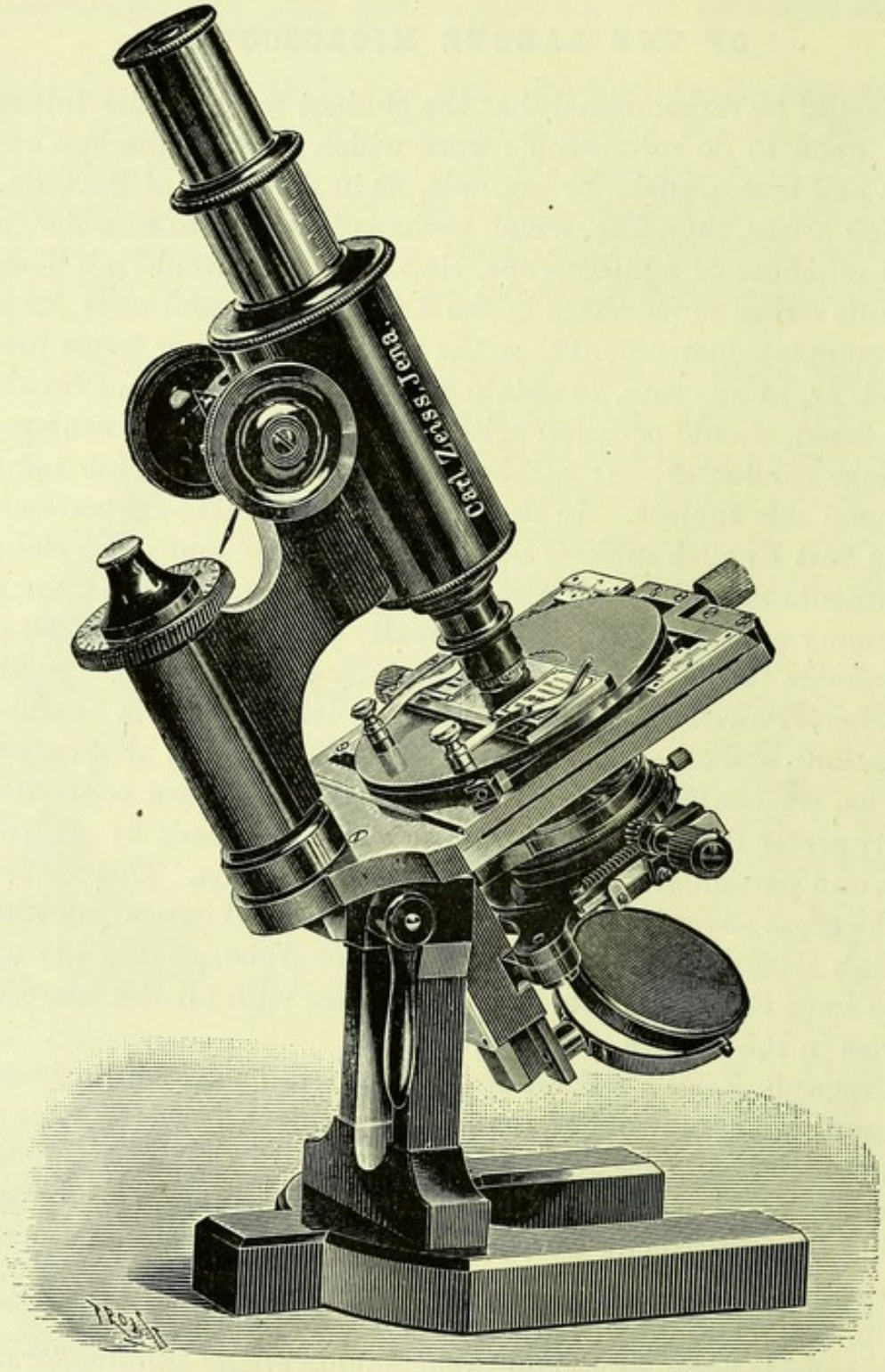

Fig. 14.—Zeiss's No. I. stand, with large stage, and short and wide body, suitable for photo-micrography.

For all practical purposes Stand IV.*a* (Fig. 15) answers all requirements, and is the form we recommend; it should be fitted with oculars 2 and 4 (14s.); a nose-piece (27s.); and lenses A (24s.),

D (42s.), and one-twelfth homogeneous oil-immersion lens with numerical aperture, 1·20 and 2· Omm equivalent focal length, costing £8, the total cost of the microscope and necessary apparatus being £23-£24. Of course the cost may be almost indefinitely

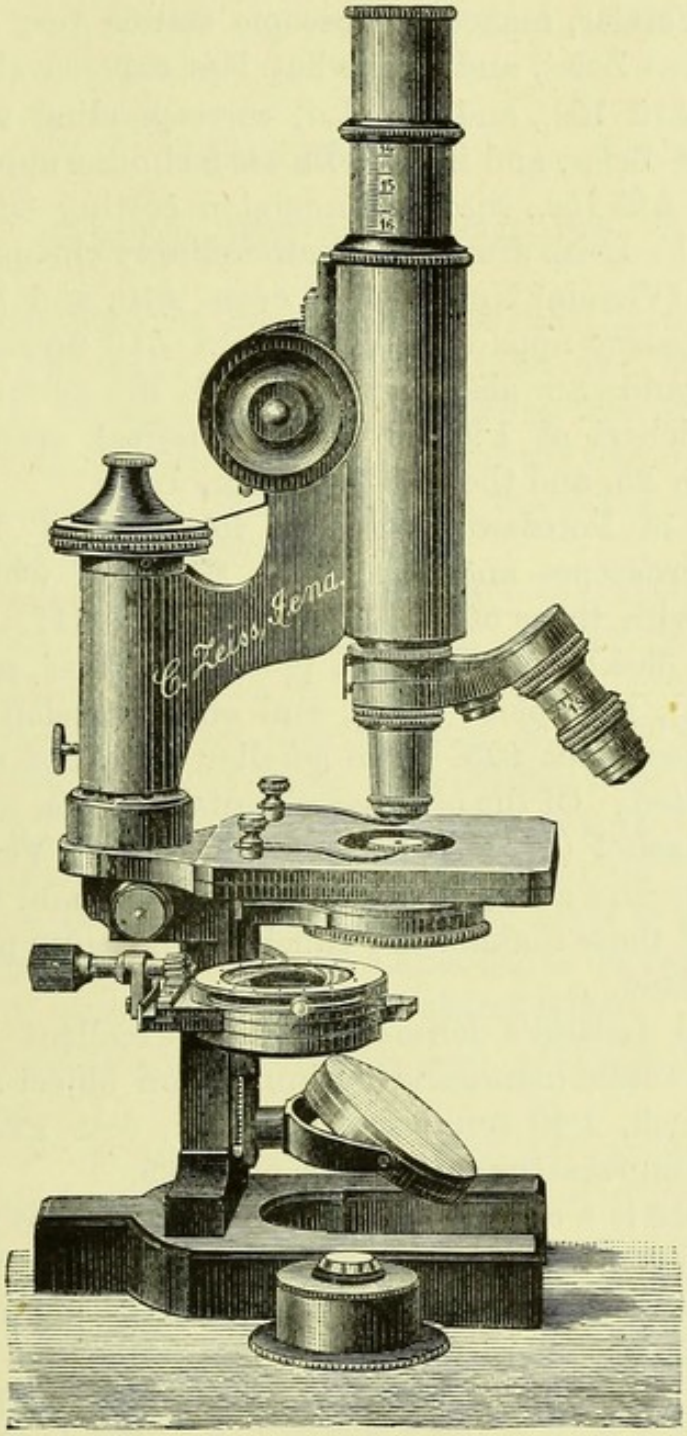

Fig. 15.—Zeiss's No. IV.*a* microscope.

increased, and the apparatus may be correspondingly improved by the purchase of apochromatic objectives and compensating eye-pieces, but these are not required except under very extraordinary circumstances. It should be added that the twelfth oil-immersion with 1·30 to 1·35 numerical aperture is more expensive than the one

with a smaller aperture, and costs £15. Some of Zeiss's small stands are good and moderate in price, *e.g.*, Stand V. 2 costs (95s.), and this, with objectives A (24s.), D (42s.), and ocular No. III. (7s.), altogether costs £8 8s.; Stand VI., with the same ocular and objectives, costs £6 13s.

Leitz, of Wetzlar, makes microscopic stands very much after the same pattern as Zeiss, and somewhat less expensively. His No. I. stand costs £12 10s., and No. I.*a*, corresponding with the above Stand IV.*a* of Zeiss, and fitted with very similar apparatus, may be obtained for £16 15s., the oil-immersion costing £5. The smaller stands made by Leitz are also correspondingly cheaper.

Reichert's (Vienna) No. I. stand costs £10, and No. II.*a*, fitted with all necessary apparatus, costs from £15 upwards. Some of his smaller stands are also very good. Of the oil-immersion lenses made by Reichert of 1·20 to 1·24 numerical aperture, the one-fifteenth costs £5, and the one-twentieth, £8 8s.

Hartnack, of Potsdam, and also formerly of Paris, supplies excellent microscopes and lenses, the prices of which correspond very closely with those of Zeiss. Stand No. VIII.*a* costs £9, and with No. IV. (30s.), and No. VII. (40s.) objectives, oculars Nos. II. and IV. (16s.), nose-piece (25s.), and oil-immersion No. I. (£10), costs altogether about £25. The smaller stands of this maker are justly celebrated. Of the other continental makers, we may mention as reliable Nachet (17, Rue St. Séverin, Paris); Verick (2, Rue de la Parcheminerie, Paris); Meyer, Enge, Zürich; and Siebert, Berlin. The stands of these makers are much on the same pattern as those already indicated.

Powell and Leland's lenses are very excellent but expensive. Their apochromatic homogeneous immersion object-glasses, $\frac{1}{8}$-inch, $\frac{1}{10}$-inch, $\frac{1}{12}$-inch, 1·40 numerical aperture, cost £25 each; and a set of three compensating eye-pieces costs £5.

A **nose-piece** is a convenient appliance screwed to the microscope tube for carrying two or more objectives, so that one may be exchanged for another without the trouble of unscrewing it from the tube. The nose-piece, represented as attached to the microscope in Figs. 2 and 15, is for two powers. It is fixed to the tube of the microscope by a screw. The arm carrying the objectives revolves on a centre, which also attaches it to the upper portion or framework of the nose-piece, which is screwed into the microscope tube at one end, and is bent away from the stage on the other. By carefully pushing one objective away from the microscope tube, the second one is brought into its place.

CHAPTER II.

HISTOLOGICAL METHODS.

HAVING now considered at some length the plan of construction of the microscope and its accessory apparatus, as well as the manner of using them, it becomes necessary to describe the various processes by means of which the structural elements of the tissues may be most effectively demonstrated. This is, indeed, one of the most important parts of the subject of practical histology.

Although as a general rule it is necessary to subject the various tissues to certain preliminary treatment previous to their microscopic examination, it should be recollected that there are certain exceptions to this rule, and that in some cases it is advisable to examine fresh tissues, without any such preparation.

The Examination of Fresh Tissues.—The simplest example of the examination of a fresh tissue would be afforded by the examination of a drop of blood. The drop is placed upon a thin piece of glass, about $\frac{5}{8}$ inch in diameter, or **cover-glass**, which is then inverted upon a glass slide or **object-glass**. The specimen of blood is at once fit for examination, and continues to be so until it dries up.

Similarly, one may examine fluid drops of any kind which are supposed to contain solid particles, the nature of which it is necessary to make out with the microscope; *e.g.*, sediments of urine, scrapings of fresh tissues or organs, or micro-organisms when suspended in water, the only requisite of success being that the particles should be thin enough to be transparent.

In some cases, again, it is necessary to examine some peculiarity of the structure of a tissue unaltered by reagents, or to observe some action, such as the movement of cilia, which speedily ceases after removal of the tissue from the body. In the examination of fresh tissues (see also p. 61) the various fluids which are likely to act upon the material may be used to keep the preparation moist; of these the most important are the following:

Normal saline solution—a 0·6 per cent. solution of sodium chloride; *i.e.*, 6 grms. in 1000 ccm.

Blood serum.

Aqueous humour.

Iodized serum—*i.e.*, serum or liquor amnii to which a trace of iodine has been added as a preservative.

It should be added that although masses of tissue just removed from the body may be frozen and cut into sections with a microtome, just in the same way as will be described later on for hardened tissues, or may be cut with a Valentin's knife without freezing, yet the sections in both cases, except for a preliminary examination, are not satisfactory, and are certainly difficult to manipulate. It is, however, sometimes of use to employ such sections of the fresh tissue for the purpose of being broken up by **teasing**. This process is done with mounted needles. These may be made very easily by taking fine-pointed needles, heating the eyes red hot in a flame, and pushing them into penholders or suitably cut pieces of wood. More elaborate needle-holders may be bought of any maker of microscopic requisites.

The method of teasing may be thus described: Cut off a small portion of the tissue to be teased with a sharp knife or pair of scissors, and place it on a clean glass slide, in a small drop of the fluid in which it is to be mounted. Place the slide upon a firm support as close to the eye as convenient, with a blackened plate of glass intervening if the object is colourless, and with a white porcelain tile or piece of white paper instead if the object is coloured, and with a couple of mounted needles separate the tissue into, as nearly as possible, parallel fibres. Some tissues cannot be so separated, and in that case they must be broken up with needles into minute pieces. Sometimes the aid of a lens or of a dissecting microscope has to be called in.

A rougher method than teasing may occasionally be used when epithelium cells have to be examined. It consists in placing a scraping of the epithelial surface on a slide, adding a drop of glycerine, and gently tapping the cells with the smooth end of an ivory or bone penholder until they are seen under the microscope to be separate. This method is, however, almost sure to damage a certain proportion of the cells. Another method, which is sometimes necessary, and is often a preliminary to teasing, is that of **softening, or dissociating.** This process is employed for two purposes — either to facilitate the teasing of tissues into their structural elements, or so that sections of tissues otherwise too hard for the knife may be conveniently cut. For either of these purposes

small pieces of the given tissue should be allowed to soak in one of the following solutions:

Potassium Bichromate, 2 per cent.—This is particularly useful for the purpose of dissolving up the cementing material between the fibres of tendon.

Baryta solution may be used for a similar purpose.

Iodized serum.—Serum with enough iodine in it to make a deep sherry-coloured solution.

Weak methylated spirit, 1 in 3, may be used for lymphatic glands, spleen, and testicle.

Chromic acid, 5 per cent., to which a few drops of sulphuric or nitric acid have been added, is the best solution for softening bone and teeth.

Hydrochloric acid, 2 per cent. to 3 per cent., or a mixture of this with nitric acid, 1 per cent., may be also used for bone, teeth, and cartilage.

Picric acid in a saturated solution is strongly recommended by some for the preparation of teeth, and also for the preparation of the cochlea; previous to cutting a few drops of hydrochloric acid may sometimes be added with advantage.

Osmic acid, in solution, 1 per cent. to 0·25 per cent., is also a good reagent for this purpose, and causes less change in the elements of the tissue than many of the above fluids.

The Examination of Hardened and Prepared Tissues.—Speaking generally, it is impossible to make out thoroughly the structure of a solid tissue until a thin section of it has been obtained. The consistence of the tissues in the recent state, as a rule, does not allow of such sections being cut, and it is, therefore, necessary to harden them before attempting that process.

The hardening of tissues may be accomplished either by freezing them—which, as has just been mentioned, is not the best way of proceeding—or by various chemical reagents. The chemical hardening reagents act either by coagulating the albumen, by withdrawing the water, or in some instances, perhaps, by combining with the albumen to form a harder compound—in a manner comparable to the process of tanning.

HARDENING REAGENTS.

The process of hardening must be attended to with the greatest attention. The choice of a suitable reagent in each case will be found, in spite of anything said to the contrary, a matter of con-

siderable moment; the method of using the reagent is equally important. The most serviceable hardening reagents are the following:

Chromic acid and spirit.—A mixture of chromic acid and spirit is the fluid recommended for general use, as it can be employed almost universally. It is thus prepared: Chromic acid, $\frac{1}{6}$ per cent. solution (*i.e.*, containing 1 grm. of chromic acid in 600 cc. distilled water), 2 parts; methylated spirit, 1 part. Or, according to another formula, equal parts of 0·5 per cent. solution of chromic acid and of methylated spirit. Either mixture produces its effects in about seven to ten days.

Chromic acid, without the addition of spirit, in 0·25 to 0·5 per cent. solutions, is a rapid hardening fluid. The tissue is sufficiently hard in a week for sections to be made; if it remains in the mixture for a longer time, there is a tendency for it to become brittle.

Potassium bichromate, in solutions varying in strength from 1 per cent. to 5 per cent. Generally speaking, a 2 per cent. solution is used, and this, if changed every four days, hardens tissues in a fortnight.

Ammonium chromate, in 2 per cent. to 5 per cent. solutions, is superior in some ways to the similar potassium salt. Tissues should not remain in it for more than a day or two.

Ammonium bichromate is specially recommended for hardening the brain and spinal cord, in solutions of 5 per cent. strength. It must be prepared afresh when wanted.

Müller's fluid is exceedingly popular as a hardening reagent. It is made by taking potassium bichromate, 2 grms., and sodium sulphate 1 grm., and dissolving in 100 cc. of distilled water. The process of hardening is slow by means of this fluid, but efficient. It has the advantage of being exceedingly penetrating, and so will harden satisfactorily larger pieces of tissue than other similar agents. An equal quantity of copper sulphate (**Erlicki's solution**) may be substituted for the sodium sulphate, and renders the fluid much more potent.

Picric acid, in saturated solutions, or a saturated solution mixed with an equal quantity of methylated spirit, or with an equal quantity of 5 to 10 per cent. hydrochloric or nitric acid, is a rapid and useful hardening fluid. It is now preferred to all other solutions by some experienced histologists.

Mercuric chloride, in solutions varying from 1 per cent. to 0·1 per cent., or mixed with acetic acid, is becoming a popular hardening reagent. Its action is rapid.

Nitric acid, 3 per cent. to 10 per cent., is recommended for hardening the brain. It requires for its action two or three weeks.

Methylated spirit is a hardening reagent in very common use, and may be employed with advantage in the preparation of salivary glands, stomach, intestine, etc.

Absolute alcohol of specific gravity 0·795 is the most rapid hardening fluid. It is said to have a greater tendency to produce shrinking of the tissue hardened than preparations of chromium.

Chromic, acetic, and osmic acids, combined in various proportions, are now much recommended. A mixture, made up as required (it will not keep well) according to the following formula, will be found of advantage: Chromic acid solution, 1 per cent., 15 parts; osmic acid solution, 2 per cent., 4 parts; glacial acetic acid, 1 part. Or a mixture of osmic acid, 0·1; chromic acid, 0·25; water, 100; without the acetic acid, is much relied upon by some. (See also p. 48.)

Directions for Hardening.—As it is necessary to use fresh tissue only, the material employed must be taken from animals just killed; it is seldom possible to obtain specimens from the *post-mortem* room sufficiently fresh to give satisfactory results.

The tissue should be cut into pieces with a sharp knife or razor. The size of the pieces should vary with the reagent used. When chromic acid is the hardening fluid, the pieces should not be larger than a small hazel-nut. When alcohol, potassium bichromate, or Müller's fluid is employed, they should not exceed twice that size.

The pieces should not be washed with water; if it be necessary to get rid of any foreign body, a small stream of normal saline solution, or of dilute spirit, or of a weak solution of potassium bichromate, should be allowed to flow upon the tissue from a wash-bottle.

The cut pieces should be placed in a large excess of the hardening reagent in a stoppered bottle.

The hardening reagent should be changed frequently—*e.g.*, the chromic acid and spirit solution on the second, fourth, and seventh days.

In all cases, in a week to ten days the specimens should be removed to spirit, or, if that fluid be contra-indicated, to a 0·5 per cent. potassium bichromate to complete the hardening.

SECTION CUTTING.

Having hardened the material, the next thing is to cut *thin* sections from it; for unless the sections be thin, no amount of after preparation will make them fit objects for microscopical investigation. The methods which have been proposed from time to time to effect this object are numerous, but they may be divided into two classes, viz.:

(1) Methods of cutting by hand.

(2) Methods of cutting with machines called microtomes.

1. Section-cutting by Hand.—In order to cut sections of a small piece of tissue it is customary to embed it in some other tissue, or in a wax mass of some kind. For the former purpose, the tissue to be cut is placed between two pieces of hard liver, or material of similar consistence, and held tightly in place between the finger and thumb, and cut with a razor in the manner to be described below. Instead of the liver, pieces of turnip, carrot, or potato may occasionally be substituted.

The usual method, however, is to embed the specimen in a wax mass.

Embedding materials are wax masses of some kind, modified according to the state of the weather and the material to be cut. The following are those most commonly employed:

White wax and olive oil, equal parts; melted and well mixed. This mass may be varied in consistency by varying the amount of the olive oil used. Japanese wax is about one-fourth the price of English, and answers almost as well.

Paraffin and Lard.—Take five parts by weight of solid paraffin (a paraffin candle will do very well), and one part by weight of hog's lard and of paraffin oil; melt at a gentle heat, and mix thoroughly. Paraffin wax (1s. 4d. per lb.) of two kinds, melting at about 43° and 55° C., may, however, now be obtained; and, by mixing in various proportions, will supply wax of required melting-points for the different seasons of the year, without admixture of lard.

Spermaceti and Castor Oil.—Four parts of spermaceti are melted down with one part of castor oil.

Cacao butter alone or combined with paraffin, wax, and oil, or with spermaceti and paraffin may be used.

To melt the Wax Mass.—The wax mass is melted in a small porcelain capsule provided with a handle, over the flame of a Bunsen's burner or spirit-lamp. Care must be taken that the material is not burnt. It is usual to place the capsule on a piece of

fine iron gauze on a tripod, and to place a gas flame from a Bunsen's burner of the smallest size beneath it; a glass rod may be used as a stirrer.

To embed the Specimen.—A piece of stout paper is taken, six inches long and three broad. This is doubled into three longitudinal folds; after this from each end folds of two inches long are marked off. The paper is then opened out, and of the three longitudinal folds the middle one forms the bottom, and the lateral ones the sides of the paper box. The ends are made from the middle part of the end folds. The ends of each flap are marked off into two equal squares, E C, C D, E^1 C^1, C^1 D^1. The squares E B A C, and E^1 B^1 A^1 C^1, are doubled into two parts across the diameters A B, A^1 B^1, and these triangular folds thus made are

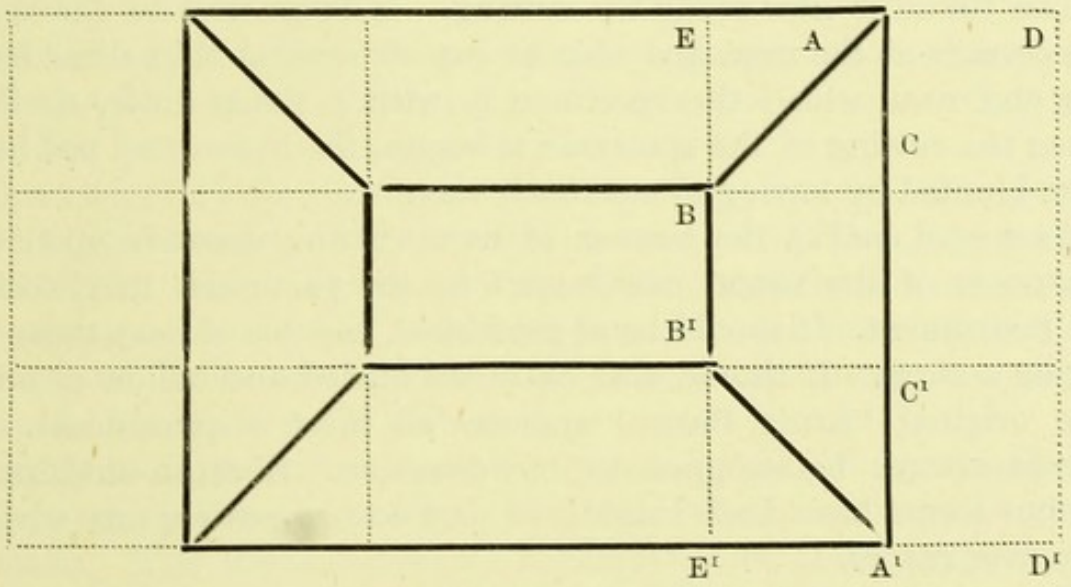

Fig. 16.—Diagram to show Formation of Embedding Box.

pinched up and pressed against the end of the box to support it; they are retained in position by the remainder of the end fold represented by A A^1, D D^1, being turned back over them.

Having made a paper box in the manner above described, and having melted the wax mass, the specimen to be embedded upon a needle mounted in a holder, and having removed the superfluous absolute alcohol (in which the tissue ought to have been immersed for at least ten or fifteen minutes before the operation is commenced) with blotting-paper, half fill the paper box with the melted wax mass, and dip the specimen into it several times, until it is thoroughly enveloped in wax. Allow the wax to cool, and place the tissue on it in the box at one end; then fill the box with melted wax, and after it has hardened, mark on the outside the position of the tissue. When quite hard, turn out the wax and the embedded tissue by

opening the ends of the box, and place for a few minutes in methylated spirit.

Instead of the simple paper box, tin boxes with removable bottoms may be substituted, or hollow brass cylinders of various diameters, placed upon a glass plate.

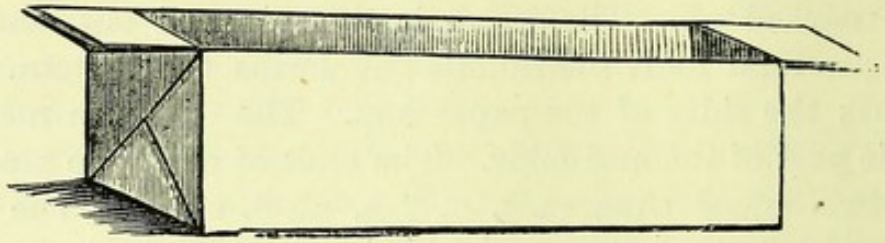

Fig. 17.—Paper Box for Embedding.

The tissue is now ready for cutting. It is as well to shave off the corners of the wax, and also to cut off several thin slices from the end near which the specimen is with a sharp knife, so that when the cutting of the specimen is begun, the razor may not have been blunted by having to cut much wax.

As a good deal of the success of hand-cutting depends upon the sharpness of the **razor**, one cannot be too particular in choosing the instrument. It should be of good steel, capable of easy sharpening on a strop. It should also be broad bladed and hollow-ground. The original 'Army Razor' answers all these requirements. It should always be stropped in one direction. **Section-cutters** of various forms have been introduced, but do not possess any advantage over razors.

Directions for Hand Section-cutting.—Be sure the razor is very sharp. Hold it firmly in the right hand, with the fingers closed above the handle; take the wax mass between the index-finger and thumb of the left hand, support the back of the razor on the former, and cut from left to right, and from heel to tip of the razor.

Let the handle be kept in line with the blade.

Keep the blade well wetted with spirit with a camel's hair brush, and float the specimens when cut into a glass capsule filled with methylated spirit.

2. Section-cutting with Microtomes.—It must be confessed that hand section-cutting is very much going out of fashion. It is now scarcely ever required except at some examinations in practical histology.

The reason is not far to seek. The skill required for hand-cutting in order that uniformly thin sections may be cut with certainty is

very rare. Microtomes by various mechanical means do away with the uncertainty of hand-cutting. They are invariably used when a large number of sections is required. Some histologists still, however, prefer hand-cutting for all delicate materials.

Ranvier's is the simplest microtome. It consists of three hollow brass cylinders, fitting one inside the other; to the most external a flat circular brass plate is fixed at one end, and to the other a cap which can be screwed on, carrying through its centre a fine screw. The tissue having been embedded in paraffin-wax in one of the cylinders and allowed to cool, the cap is adjusted, the microtome held in the hand, and the screw turned until the wax is carried up sufficiently high for the embedded specimen to be cut; the flat plate guides the razor. After each section is cut, the screw is turned slightly, thereby presenting sufficient of the embedded tissue for another section.

Stirling's microtome is on the same principle as Ranvier's, but is larger, and is fixed to a table by means of a screw. Only one cylinder, however, is fitted to the instrument.

Rutherford's microtome provides a trough which may be used to contain a freezing mixture of ice and salt. When this method is used, the tissue is embedded in thick gum, which, on freezing, becomes a solid mass, in which the material may be readily cut.

Both of these microtomes may be provided with glass plates instead of brass to guide the razor.

In these three microtomes the tissue embedded in its paraffin or gummy cylinder is raised to the knife. This would seem to be a mistake. The tissue should be firmly fixed, or at any rate should be exposed to as little disturbance as possible, and the razor should be lowered to it, and not *vice versâ*.

In **Zeiss's** microtome (Fig. 18) the objection to the above microtomes is partly met by the cylinder containing the wax being moved upwards bodily, and not the wax only. The instrument is well made, and the screw is so fine that sections of extreme thinness may be cut by its aid. Its price is £2.

This microtome consists of a circular plate of metal, upon which is cemented a piece of thick glass, supported upon a heavy base of brass by two pillars. The metal and glass plate are perforated in the centre by an aperture about an inch in diameter, into which fits a brass cylinder in which the specimen to be cut is embedded in wax. This cylinder is capable of being raised by means of a screw passing upwards from the centre of the base-support.

In order to work with this microtome, this screw should be screwed home by turning the milled head; the central brass

cylinder should be pushed down until the projecting point of the metal button which is fixed into its lower end touches the centre of the upper surface of the milled screw-head; melted wax and oil mixture should now be poured into the cylinder until it is filled, and should be allowed to become cool and hard.

Above the brass cylinder there is left a certain interval; the upper edge of the cylinder not being flush with the upper surface, this interval forms a shallow cup about half an inch deep, and in it the tissue is embedded in hot paraffin after having been soaked in the same material for a couple of hours previously. When the paraffin is cool and hard, by the turning of the screw below it is slowly protruded above the edge of the circular aperture in the cutting-plate; when by successive turns of the screw the tissue

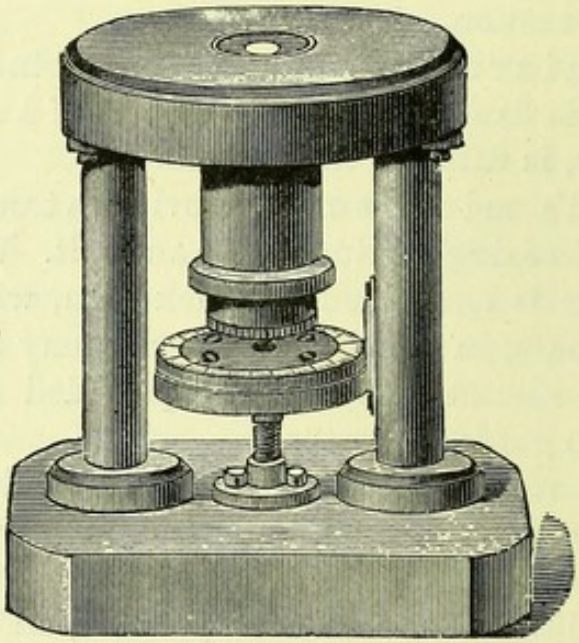

Fig. 18.—Zeiss's Microtome.

is reached, the projecting paraffin may be cut off by sweeping the razor, well wetted with methylated spirit and guided by the cutting plate, through the mass. In a similar manner sections of proper thinness may be cut by turning the screw through as many graduations as are sufficient between each sweep of the razor.

Freezing Microtomes.—The principle upon which these machines work is very simple. The specimen to be cut is saturated with gum; the gum is then frozen, and with it the contained material and sections of the frozen mass are then made. The most commonly used form of freezing microtome in this country is known under the name of **Williams's** microtome, and is made by Swift, Tottenham Court Road (Figs. 19 and 20).

In the original form of this microtome (Fig. 19) the freezing agent

employed is a mixture of ice and salt. In the second form (Fig. 20) the ether spray aparatus is employed to procure the requisite freezing temperature.

The apparatus (Fig. 19) consists of a circular wooden box eight or nine inches in diameter. Into the centre is fixed a circular solid brass pillar, with a brass plate at the top. This box contains a freezing mixture of ice and salt, and is provided with a waste tube to get rid of the water as the ice melts. The cover of the box is wooden, but has a plate of glass cemented upon its upper surface;

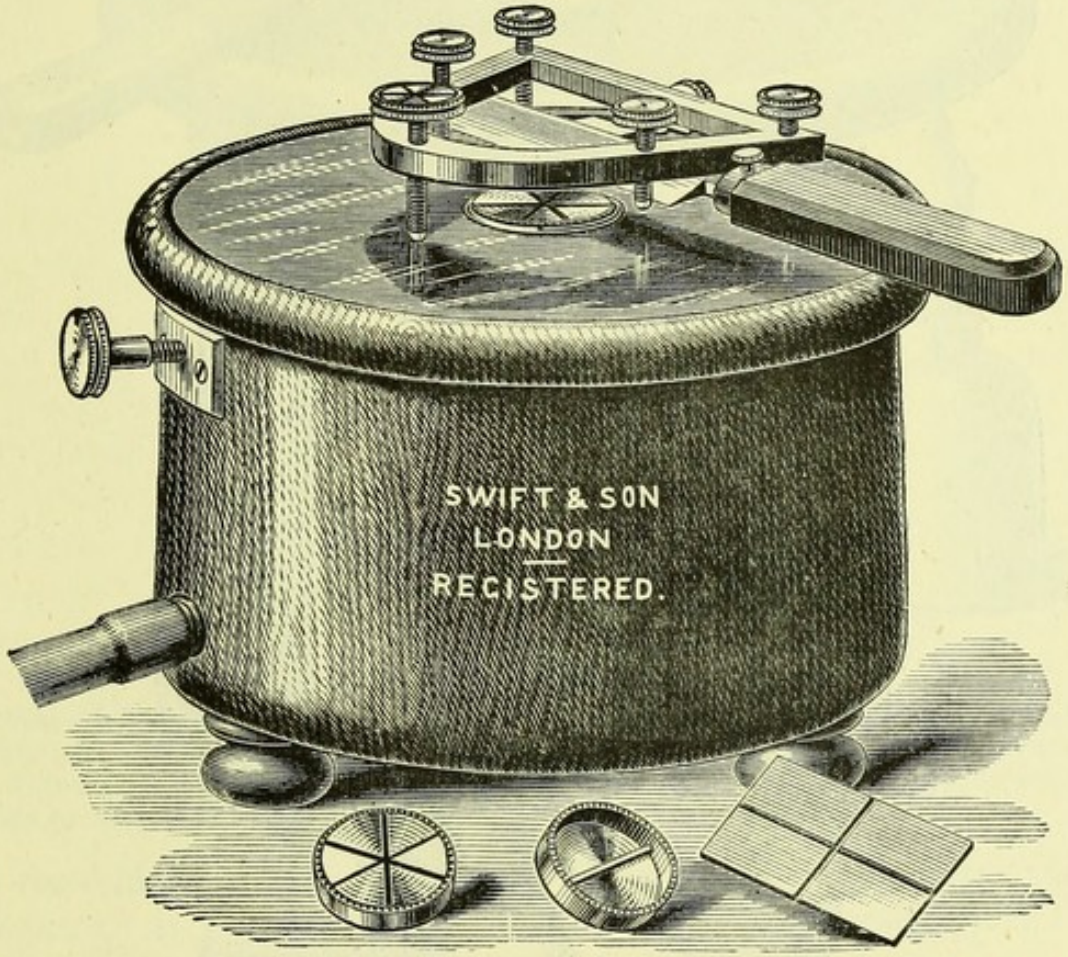

Fig. 19.—Williams's (Ice and Salt) Freezing Microtome.

it also has a central aperture to admit the top of the brass cylinder. This cover is fixed down after the freezing mixture has been placed in the box, and fastened with a lateral screw. The specimen, which should first be left for twelve hours in water (to get rid of the spirit), and then for two or three hours in a solution of gum, is afterwards placed on the cylinder plate in a little of the gum. The gum is soon frozen, and the specimen is thus fixed. The cutting is effected by means of a razor, which is fixed in a movable triangular brass frame, supported on three screws. By the adjustment of the front screw the thinness of the section is regulated. The frame works

smoothly over the glass table. The upper surface of the razor should be slightly moistened with gum. As the sections are cut they should be swept off the razor with a camel's-hair brush, and should be washed in warm water before staining.

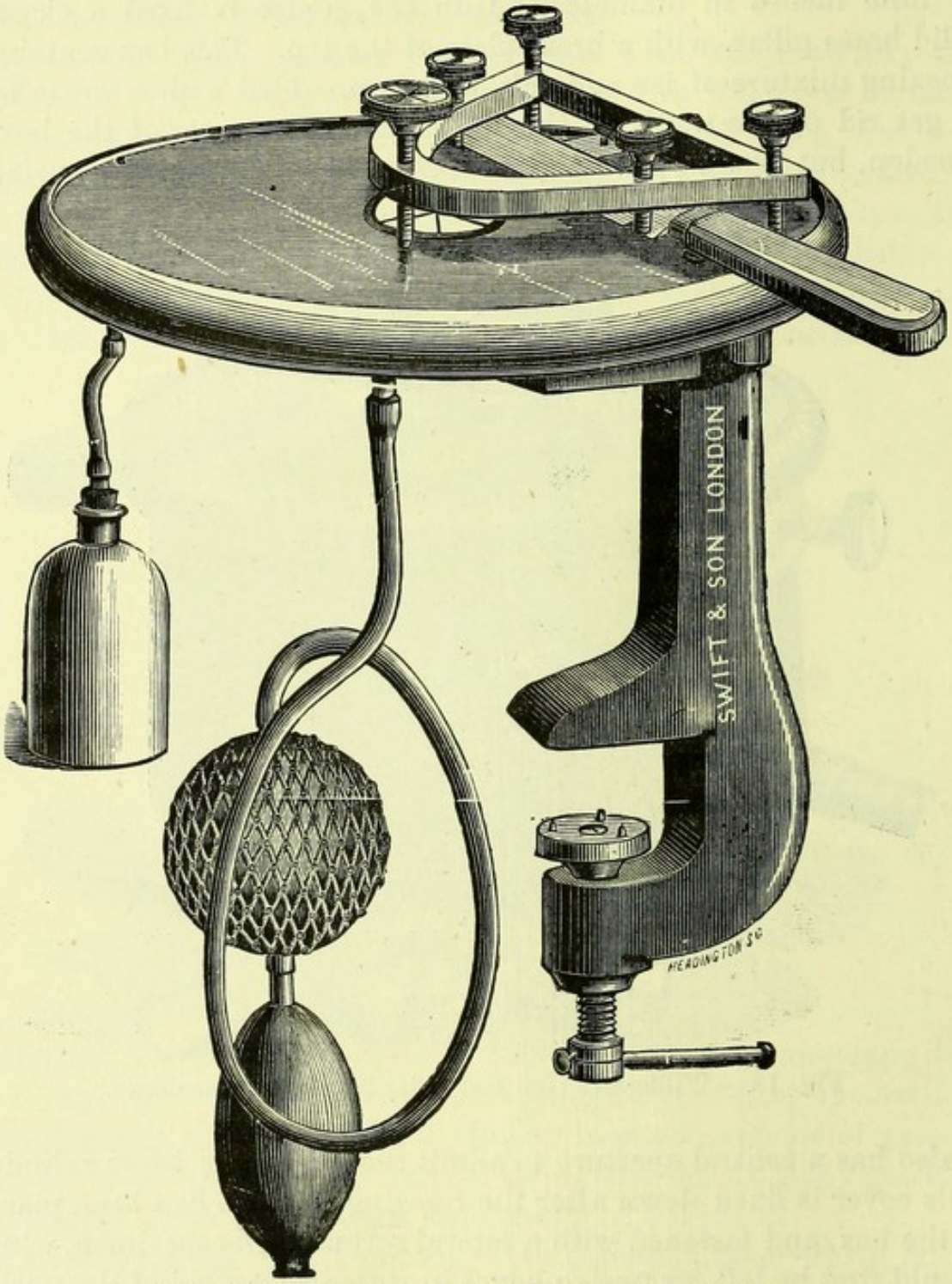

Fig. 20.—Williams's (Ether) Freezing Microtome.

The microtome adapted for use, with **ether** as the freezing agent, is now much used. The cost of these microtomes varies from £2 15s. upward. A very simple and useful modification of this ether freezing microtome is made by Frazer, of Edinburgh.

Freezing mixtures are made by taking pounded ice,

adding some rough bay-salt, and thoroughly incorporating the one with the other. In very cold weather snow may be obtained and used in place of the ice.

A strong **solution of pure gum arabic** in boiling distilled water is made, and filtered through calico. To this is added a few drops of melted pure carbolic acid. Some advise the addition of white sugar, in various proportions, to the mucilage. The proportion of gum is about a pound to a quart of water. It should be kept in a stoppered bottle, and boiled occasionally, to prevent decomposition.

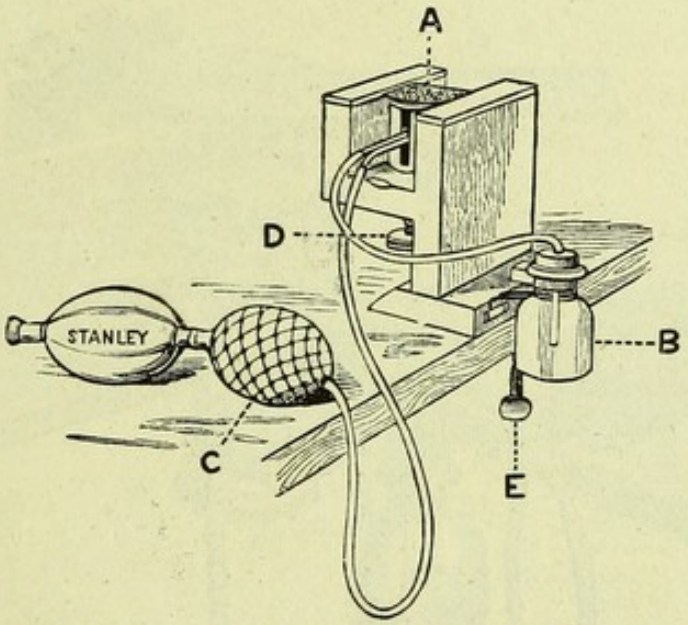

Fig. 21.—Cathcart's Microtome.

A, Metal plate upon which the tissue is placed in gum; B, Bottle containing ether; C, Ether spray apparatus; D, Screw to raise tissue when frozen; E, Screw to fix microtome to table. The cutting is done by a plane which rests on the supports on either side of A.

A convenient and cheap form of ether freezing microtome, viz., Cathcart's,* is represented in Fig. 21; its employment requires a certain amount of practice.

Other ether freezing microtomes may also be mentioned. In Fig. 22 is seen an engraving of Katsch's ether freezing microtome. The method of using will be explained below. The cost of the microtome is £4 17s. It is a very handy instrument, and is extensively employed on the Continent.

A somewhat similar instrument is made by Schanze, of Leipzig, also of considerable practical value; it offers some advantages over Katsch's instrument. It is made in four sizes, the cost of which complete is respectively £8 15s., £6, £5, and £4 5s., according to the length of the plane along which the metal wedge bearing the razor can travel, viz., 40 cm., 25 cm., 20 cm., and 17 cm. A

* The cost of Cathcart's microtome is from 15s. upwards.

microtome made by Zeiss, of Jena, after Körting, is very similar. Its cost complete is £7 12s. 6d.

A very excellent microtome, and one which can be well recommended, is that by Reichert, of Vienna (Fig. 23). The principle of action is much the same as of those microtomes before described. The razor is carried by a heavy metal wedge, which works with little friction in an angular slot. There is an automatic action for raising the embedded material. The cost of this microtome complete for cutting both frozen and embedded materials is about £7.

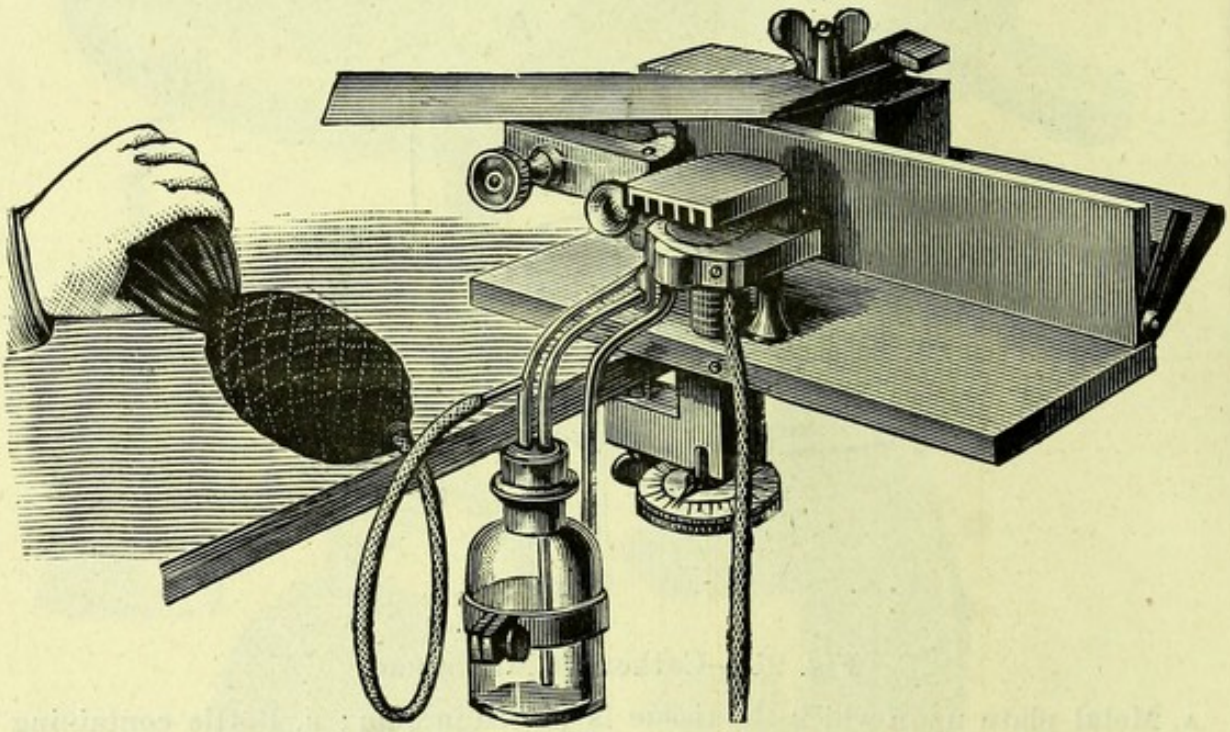

Fig. 22.—The Ether Freezing Microtome of Katsch (Munich).

The microtomes of Jung, of Heidelberg, are of great delicacy. The prices of the three sizes complete are, respectively, about £15, £12, and £8 10s.

Boecker, of Wetzlar, makes an excellent microtome for £9 9s., and Leitz, of Wetzlar, one which costs, complete, £7.

Meyer, of Enge, Zürich, supplies a microtome, made on much the same principle as Reichert's, in three sizes, complete, £9 10s., £8, and £6 respectively.

Directions for using any microtome are only required by a beginner; in a short time practice will entirely supply their place. The chief thing necessary for success in cutting sections is, as H. Kühne has well put it, to adopt any one form of microtome, and then to get a complete mastery over it by constantly using it and no other. The following is an epitome of the method for using Katsch's microtome, given by the same author. These directions are very practical and clear.

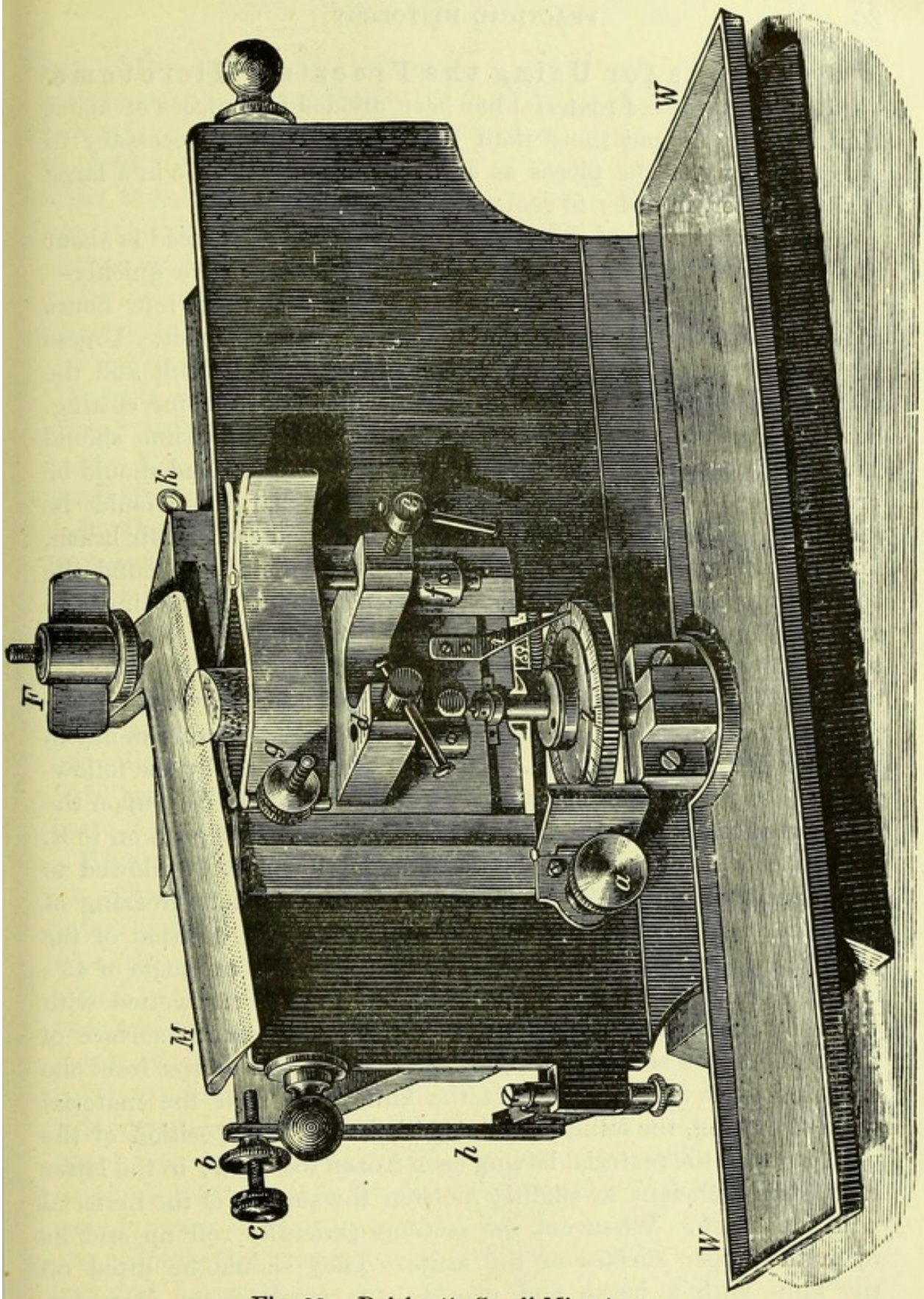

Fig. 23.—Reichert's Small Microtome.

, Razor carried on a solid metal block, K. F, Clamping arrangement for the razor; by knocking against the screw *c*, fixed by milled head *b*, the razor carrier, by means of a spring arrangement *s*, causes a revolution of the wheel *z*, and so raises the object to be cut held in the object carrier *g*, which is fixed at *f* by a small vice. It can also, when necessary, be inserted into *d*; and *i* is the indicator of the amount of the raising of the object which takes place each time the spring arrangement *c* is set in action.

Directions for Using the Freezing Microtome.—After the hardened material has been divided into pieces of about 2 mm. thick and not more than 10 mm. long, it is necessary to place as many of the pieces as are to be cut at one time in a large basin of water, in order to remove the alcohol.

If the temperature of the water in which they are placed is about 37·5° C., the extraction of the alcohol takes place more quickly—viz., in an hour—whereas, if cold water is used, three or four hours at least are required for the thorough removal of the spirit. Unless the alcohol is thoroughly removed the freezing is difficult and the thawing is so quick as to allow hardly sufficient time for cutting. The material when placed upon the plate of the microtome should present, if possible, an angle to the edge of the knife, and should be pressed down with a small weight. The margins should be moistened with water by means of a long-handled camel-hair brush, and the moisture should extend for some little distance around the specimen to be cut, in order that the exact moment of freezing and thawing may be observed. It is essential to apply the ether spray so exactly that *too low a temperature*, whereby *the material is rendered too hard for cutting*, is not reached; for only just below the freezing-point is the temperature such that the consistency of the material is fit for cutting. This point is attained by the following method: The ether spray first of all is allowed to play upon the under surface of the plate until the material is firmly frozen on to it. The water which surrounds the tissue upon the plate is allowed to thaw, and then the spray is applied and the complete freezing of the material is accomplished. After regulating the position of the plate, the metal block, to which the knife is fixed at an angle of 45°, is firmly grasped, the upper surface of the knife being wetted with water by means of a camel-hair brush, and a smooth surface of the frozen tissue is cut by bringing the razor and its carrier from one end of the slot to the other. If the knife slips over the material without cutting, the failure depends either upon the position of the plate or upon the material having been frozen too hard; in the latter case, it is sufficient to slightly moisten the surface of the material with the brush. When cut the sections generally roll up and lie upon the upper surface of the knife. They should be lifted off the knife with a brush and placed in a small basin of water. Whilst the right hand manages the knife and the brush, the left attends alternately to the raising of the plate and to the spray. To regulate the latter is one of the most difficult but at the same time one of the most important parts of the whole process. As soon as the water upon the plate begins to thaw, three to five

jets of the spray suffice to freeze it again and to make further cutting possible. Too hard material, however, produces complete failures. The sections can be made of the utmost thinness if such be desired. With some material, however, sections may be too thin; thus, for instance, sections of intestine which are too thin do not stand the transference from the alcohol into the aqueous solution of the dye, as the mere diffusion streams which are produced under such conditions are sufficient to tear them, and this may occur with all loosely-connected material. In such cases, therefore, the sections must be cut rather thicker. Not only the slipping of the knife but also unequal thickness of sections, by which they appear striated, arises from the material having been frozen too hard. The sections are in the latter case apt to break asunder in small streaks.

Another method of preparing tissues for section cutting must now be described, viz., the **saturation method.** It consists not only of embedding the specimens to be cut in some supporting material, but also of thoroughly saturating them with it so that the parts of a delicate tissue may be thoroughly held together and retained in their normal relation to one another when cut into thin sections. Two substances may be used for this purpose, viz., **celloidin** (or collodion) and **paraffin.** Several methods of employing them have been proposed. The following plan will be found to answer best:

The Celloidin Method.—The pieces of material to be cut, which should not as a rule be larger than dice, and previously properly hardened, should be placed in a well-stoppered bottle of *absolute alcohol* for twenty-four hours, and should then be transferred to a second bottle, containing a *mixture of equal parts of absolute alcohol and methylated ether*, for twenty-four hours. The pieces of material are then ready for placing in the *celloidin* solutions*, first of all into a *thin solution* of about the consistence of thin syrup, for twenty-four hours, and next in a *thicker solution* of about the consistence of white of egg for twenty-four hours. These solutions may be made by dissolving as much celloidin as is necessary for each in equal parts of absolute alcohol and ether. After the celloidin has thoroughly penetrated, the pieces should be taken out and mounted one by one on stout corks, allowed to remain exposed to the air for about ten minutes, and should then be placed for twenty-four hours in methylated spirit and water, equal parts. All the steps may be hurried somewhat, and twelve hours in each

* Celloidin is sold in chips in cardboard boxes at about 4s. the ounce.

solution is no doubt sufficient; but the success of the operation is more certain if the time allowed is longer. Instead of celloidin, many workers employ collodion. It is well to have four bottles, labelled Nos. 1, 2, 3, and 4, containing the required solutions, so that the material can be transferred from one to the other without loss of time.

In order to cut sections of the celloidin-embedded material, one of the already-described microtomes may be used, of which, perhaps, Reichert's is the most suitable for the purpose. In cutting, the razor must be well wetted with spirit, and the sections when cut must be swept off the knife with a camel's-hair brush and placed in distilled water. The after-treatment of these sections will be described on p. 55.

The Paraffin Process is as follows: The pieces of material are, as in the celloidin process, first placed for twenty-four hours in *absolute alcohol;* next in *anilin oil* for twenty-four hours; next in *xylol* for twenty-four hours; next in *paraffin dissolved in xylol* for twenty-four hours; and finally in *paraffin* at a temperature of 40° to 50° C. for from twelve to twenty-four hours. After this the pieces are taken out of the hot paraffin, and may then be embedded in the same material in a metal or paper box, such as is

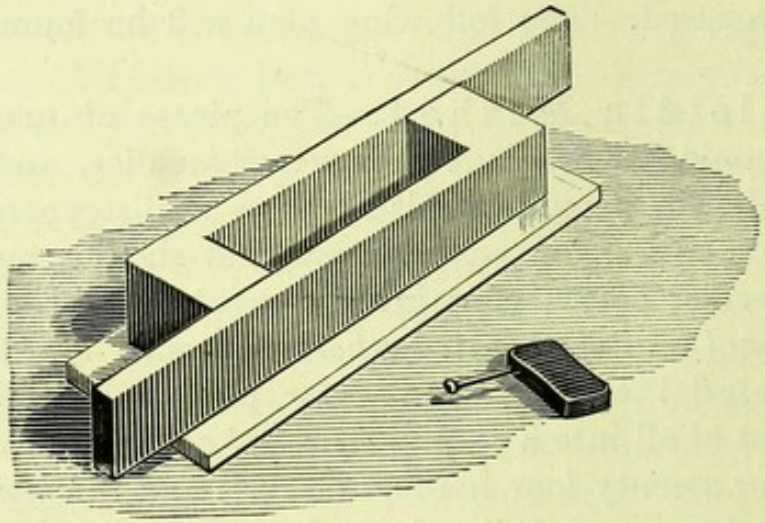

Fig. 24.—Embedding Trough.

described on page 27, or in one formed by two L-shaped pieces of metal placed together upon a glass plate, as in Fig. 24. When quite cold the superfluous paraffin may be cut away with a scalpel, and the material surrounded by the wax is ready for the microtome.

The paraffin-embedded material may be cut so as to form ribbons of consecutive sections by means of (*a*) *the Cambridge rocking microtome* (Fig. 25), or by (*b*) *Minot's microtome* (Fig. 26).

In using the **rocking microtome** it is necessary that the razor should be fixed, and the preparation has therefore to be advanced over its edge.

The apparatus consists of a solid cast-iron framework, with two uprights at one end, which are provided with slots at the top, into

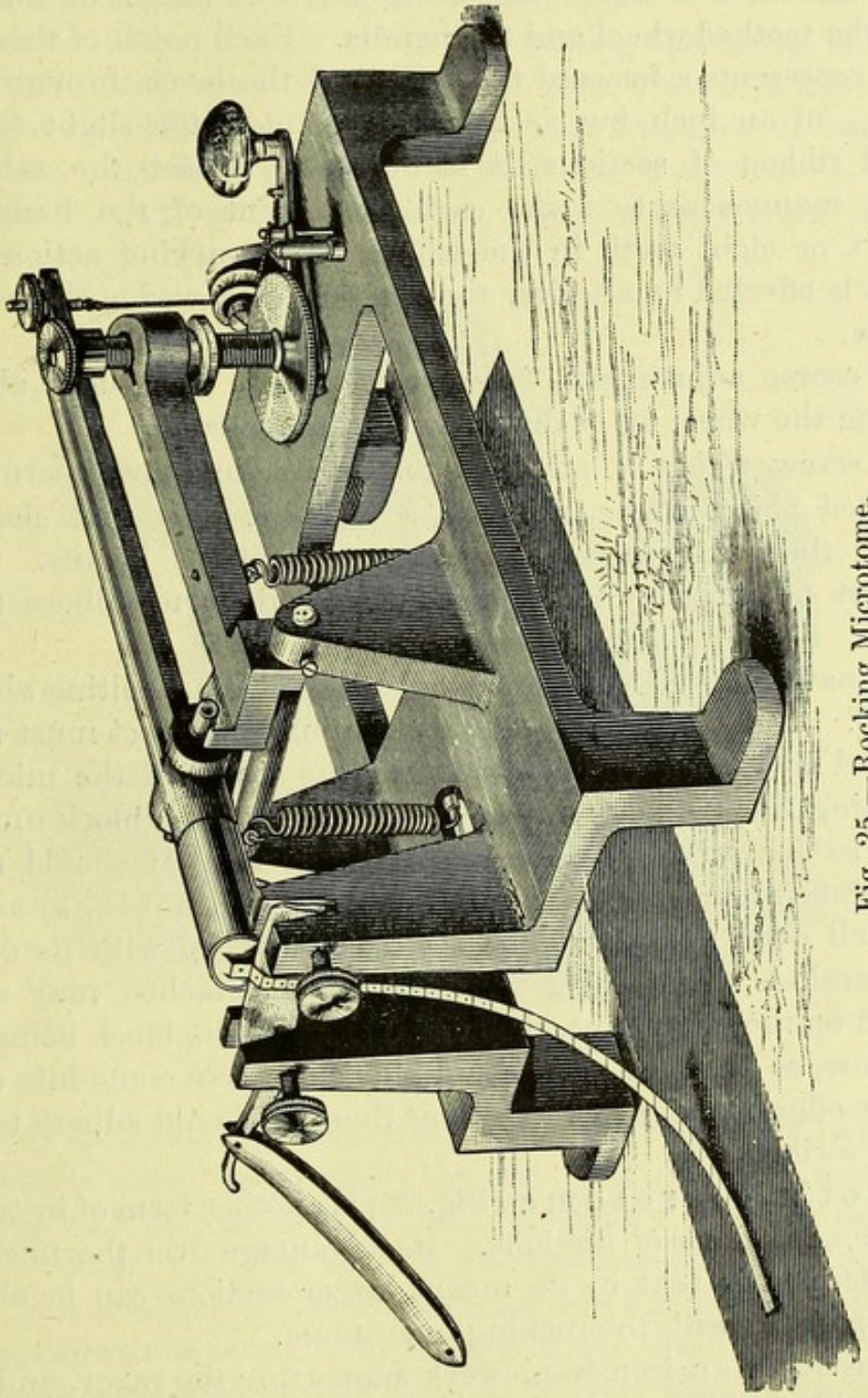

Fig. 25.—Rocking Microtome.

which the razor is fixed in position by screws. The specimen embedded in a paraffin mass of proper consistency is carried in a brass holder, placed at the end of the shorter arm of a lever rocking about a horizontal axis, whose longer arm can be depressed by a

cord passing over a pulley. This cord is attached to the handle as seen in Fig. 25. A spring brings the lever back into position as soon as the handle is released. By the up-and-down movement of the lever the specimen embedded in paraffin is cut by being brought against the edge of the razor.

The thickness of the section is regulated by means of the second lever, the toothed wheel and the handle. Each notch of the toothed wheel represents a forward movement of the lever through a space of $\frac{1}{40000}$ of an inch, but as this movement is too slight to obtain a good ribbon of sections, it is necessary to set the ratchet in such a manner as to make each movement of the handle pull over six or eight teeth in the wheel. The varying action of the ratchet is effected by altering the position of a wedge-shaped piece of brass.

The coarse adjustment of the second lever is brought about by screwing the wheel up or down the vertical pillar.

The screw which is attached to the rocking lever permits the embedded object to be adjusted so that it only just clears the edge of the razor when the cutting of sections begins. This is the most favourable condition for the sections to adhere to each other.

The material to be cut is embedded in paraffin, melting about 48° to 55° C. The block of paraffin containing the object must then be cemented to the paraffin contained in the socket of the microtome by melting the surface with a heated knife. The block must now be cut so that the opposite sides are parallel. It should now be dipped into melted soft paraffin, melting about 45° C., so as to be coated all over, and should be again squared up with its opposite sides parallel. The socket with the block attached may now be replaced on the microtome, the coated sides of the block being above and below, so that the one coated side shall first come into contact with the edge of the razor. Sections thus cut should adhere together to form a ribbon.

Minot's Microtome (Fig. 26) is another form of instrument for cutting ribbons of sections. Its advantage over the microtome just described is that by its means larger sections can be obtained than is possible with the rocking microtome.

It consists of an iron framework supporting the razor, and of an upright pillar, upon which slides the mechanism bearing the preparation to be cut. The movement of the specimen over the razor is vertical, and is effected by means of a wheel and crank. As in the previous case the thickness of the section is regulated by a toothed wheel and ratchet. The movement of the ratchet is in turn regu-

lated by a small revolving plug containing a number of stops. The shortest stop allows of the escape of a single tooth, and so of a forward movement of $\frac{1}{300}$ mm., whilst the longest permits of the escape of six teeth or $\frac{1}{50}$ mm. advancement. The plate for embedding is removable at will, and coarse movements are effected by raising the ratchet and turning the milled head.

Another and very simple method of cutting specimens saturated with paraffin, and especially adapted for large masses of material, is based upon the principle of the knife-carrying triangle. It was found in practice that the small brass caps for use with specimens embedded in wax, to be screwed on to the central brass pillar in

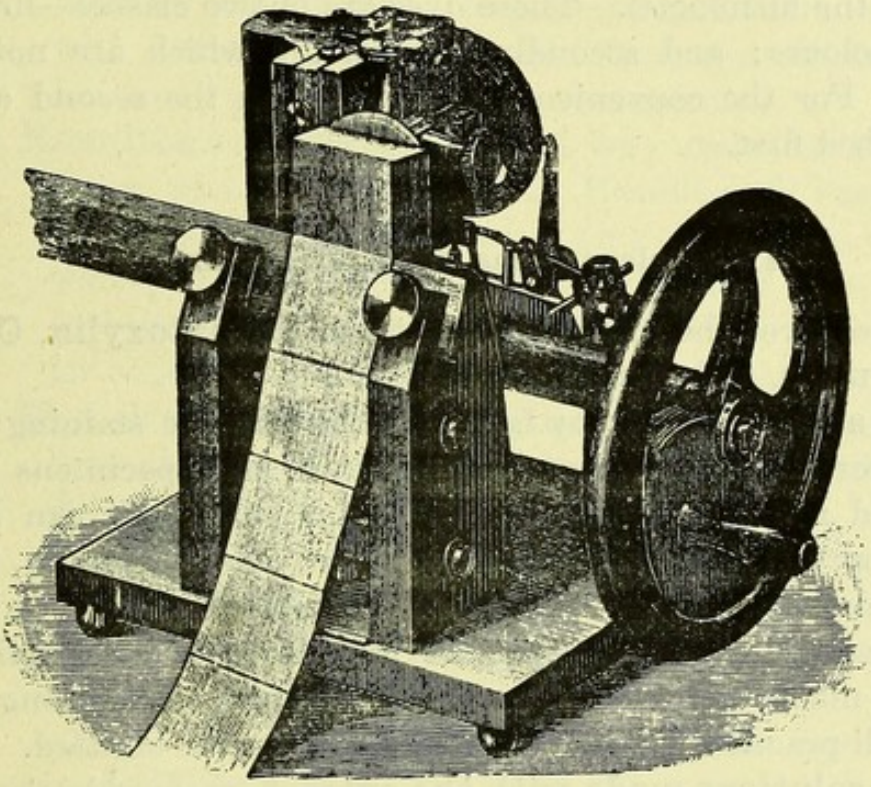

Fig. 26.—Minot's Ribbon Microtome.

Williams's microtome, did not work well. Instead of this a sheet of plate-glass is taken, upon which the specimen to be cut, thoroughly infiltrated with paraffin-wax, is fixed by slightly warming the lower surface. When the wax is cold the specimen is fixed upon the plate. The triangle in use in this case is of much heavier make, and the screws are larger, and of finer worm, than usual. With a simple apparatus such as this, sections of every kind of tissue which will bear staining in mass and soaking in melted wax may be made with ease. We have had much success with embryological specimens treated after this manner. Stanley has made these heavier triangles for us. Their cost is about £1 5s.

THE STAINING OF SECTIONS.

One of the most important operations in histological research is the process of staining. By its aid alone can the structural elements of the tissues be rendered sufficiently distinct to be properly made out and thoroughly investigated under the microscope. The method of staining varies under different circumstances, and it will be best to give an account first of all of the various staining reagents, and afterwards to describe the method of using them.

Staining Reagents.—Those dyes which have a special selective affinity for the nuclei of the tissues are the ones most useful to the histologist. These dyes are of two classes—firstly, the aniline colours; and secondly, the colours which are not aniline colours. For the convenience of description the second class will be described first.

A.—Nuclear Stains which are not Aniline.

Of these dyes the most important are **Hæmatoxylin, Carmine, Picrocarmine,** and **Cochineal.**

Hæmatoxylin is by far the most valuable staining reagent. It is a certain and intense nuclear stain, and specimens coloured by its aid retain their tint unaltered for years. It can be made either from the chips of the hæmatoxylum campechianum, from the semi-solid extract, from the purified inspissated extract, or from the isolated active principle, hæmatoxylin. Some workers still prefer the stain made by boiling the chips in alum water for long periods, but for all practical purposes this method may be omitted.

Of the **solutions made with the extract** (preferably the purified and dried form), the following can be strongly recommended. It is made by taking dried extract of hæmatoxylin, 60 grms.; alum in powder, 180 grms.; and rubbing thoroughly together in a mortar, then adding slowly 300 cc. distilled water; mixing carefully and filtering; to the filtrate adding 20 cc. of absolute alcohol, or 50 cc. of a 1 per cent. solution of thymol, and preserving in a stoppered bottle. The solution should be kept in a cool place for at least a month before using. The older it is, within certain limits, the more excellent it becomes. A second but weaker solution may be made by again adding 300 cc. of distilled water to the same logwood-alum, and proceeding in a similar manner.

Of the many **solutions made with Hæmatoxylin,** it is only necessary to mention a few.

(1) **Alcoholic Hæmatoxylin** is prepared by making saturated solutions of crystallized calcium chloride and of alum in proof spirit, mixing the solutions in the proportion of one to eight, and adding to this mixture a saturated solution of hæmatoxylin in absolute alcohol, drop by drop, until the whole becomes a dark purple. This solution may be used at once, but is greatly improved by keeping.

(2) **Delafield's Hæmatoxylin.**—A most excellent form of the reagent. It is made by taking 4 grms. of hæmatoxylin and dissolving in 25 cc. of absolute alcohol and adding it to 400 cc. of a saturated solution of alum. The mixture is then placed in an unstoppered bottle and exposed to the light for several days. It is afterwards filtered and mixed with 100 cc. of glycerine, and 100 cc. of methylated spirit. It is not ready for use under two months.

(3) **Hamilton's Hæmatoxylin.**—A very useful modification of (2) is given by Prof. D. J. Hamilton:* hæmatoxylin, 12 grms.; alum, 50 grms.; glycerine, 65 cc.; distilled water, 130 cc. Boil, and while hot add 5 cc. of liquid carbolic acid. Allow the mixture to stand in the sunlight for at least a month. It stains very quickly.

(4) **Ehrlich's Hæmatoxylin.**—Hæmatoxylin, 2 grms.; absolute alcohol, 60 cc.; glycerine, 60 cc.; distilled water, 60 cc.; glacial acetic acid, 3 cc.; alum to saturation. The solution should be kept in the light for a fortnight at least, and by that time should be of a deep reddish-brown colour. It is then filtered. It is a very quick and reliable nuclear stain, and also very powerful. Sections stained with it should be washed out in tap instead of in distilled water.

Another formula for a solution of hæmatoxylin will be given in the section of the book where its use is described.

Carmine.—This dye was formerly much more used than it is at present. A great variety of formulæ exist for making solutions of it. It may be used in alkaline, neutral or acid aqueous forms, or it may be employed in alcoholic solutions. Neutral (and alkaline?) carmine is called a diffuse and not a nuclear stain.

(1) Take 2 grms. of carmine, and rub thoroughly in a mortar with a few drops of water; then add 4 cc. liq. ammoniæ, and 48 cc. distilled water; filter into a bottle, which should be left unstoppered for a day or two for the excess

* 'Text-book of Pathology,' vol. i., p. 79.

of ammonia to evaporate. This forms a strong solution, which must be diluted before using (**Klein**).

(2) **Beale's solution** is thus prepared: Dissolve carmine grm. j. in liq. ammoniæ fort., 3 cc. warm; add aq. destillat. 120 cc., and filter. Then add glycerine, 30 cc.; and spir. vini rectif., 120 cc., and keep in a well-stoppered bottle.

(3) **Borax carmine** is made by thoroughly mixing carmine (grms. ij.) and borax (grms. viij.) in a mortar, and dissolving in warm water for twenty-four hours. The supernatant fluid, which should be decanted, is then ready for use. It is, however, best to add dilute acetic acid, and to filter.

(4) **Alum Carmine.**—Boil an aqueous solution of ammonium alum with excess of carmine for ten to twenty minutes; filter, and dilute the filtrate until it contains 1 to 5 per cent. of alum. Add a few drops of carbolic acid to prevent the growth of fungus.

(5) **Alcohol Carmine.**—A teaspoonful of carmine dissolved by heating for about ten minutes in 50 cc. of 60 to 80 per cent. alcohol, to which three or four drops of hydrochloric acid have been added, and then filtered.

(6) **Lithium Carmine.**—Carmine, 2·5 grms.; saturated watery solution of lithium carbonate, 100 cc. Stain with this for 2 to 7 minutes; wash for ½ to 1 minute with a mixture of HCl, 1 cc. to 100 cc. of 70 per cent. alcohol, and then in pure distilled water.

Picrocarmine, *or solution of picrocarminate of ammonia*, is now much used in combination with another dye when it is expedient to 'double-stain' tissues in order to bring out certain special features in their structure. It is prepared by adding a saturated ammoniacal solution of carmine to a saturated solution of picric acid until a precipitate begins to form, evaporating in a water-bath to one-third its bulk—filtering and evaporating the filtrate to dryness—a crystallized mass, easily soluble in water, is obtained, which is picrocarmine. The strength of the solution should be about 1 per cent. to 3 per cent. During preparation the ammonia should be kept in excess.

It may also be made by taking Beale's carmine without alcohol, and adding the picric acid in a similar manner. The glycerine prevents burning, which is not unlikely to occur.

Many other formulæ have been given for making this useful dye, but they possess no advantage over the ones given above.

Cochineal is a very valuable nuclear dye. It stains quickly (3 to 5 minutes), it is very selective, and particularly useful for connective tissue. It possesses the great advantage of being capable of staining sections of tissues hardened with any kind of hardening fluid. The colour may be varied by washing out in a dilute acid or a dilute alkali. It is excellent for staining *en masse*.

(*a*) Take 7 grms. cochineal and 7 grms. alum in powder, thoroughly rub together in a mortar, and add 700 cc. distilled water; evaporate to 400 cc., filter *twice*, and afterwards add ʒj. or ʒij. absolute alcohol, or of 1 per cent. thymol solution.

(*b*) Powdered cochineal, 10 grms.; alcohol, 70 per cent., 100 cc. Macerate for a week in a stoppered bottle, shake up frequently. The filtered solution will be ready for staining. Wash stained sections in alcohol of above strength.

Purpurin (Ranvier).—This dye (derived from madder-root) was first introduced by Ranvier. The most stable solution is that of Grenacher — glycerine, 50 cc.; powdered alum, 2 grms. Add a knife-pointful of purpurin, and boil. Let the solution stand for several days, and filter. Staining in this solution takes 10 to 30 minutes.

Ink-stain.—Excellent results may be obtained by staining nervous tissue, especially the spinal cord, in diluted solutions of Stephens's blue-black ink.

B.—Nuclear Aniline Stains.

An exceedingly large number of stains are now derived from *aniline* (C_6H_7N), which is one of the coal-tar products. Most of these colours are formed from two compounds of aniline, viz., *rosaniline* and *para-rosaniline*, which are made by heating together aniline and toluidine in different proportions, with oxidizing agents.

Of these stains almost all can be made to give nuclear staining by certain methods of use, but very few are, except under these conditions, so selective in their action as the stains described under A, the great majority being diffuse stains. For general histological purposes aniline colours are not so useful as the pure nuclear stains already described, neither can they stain tissues *en masse* at all

adequately, but for bacteriological and other special purposes they are invaluable.

The aniline colours are employed in *aqueous* or *spirit* solutions. They may also be used in solutions made with *aniline-water*, *i.e.*, water which, by shaking up with aniline oil, dissolves a certain amount; with *carbol-water*, a 5 per cent. solution of carbolic acid in water; or with *thymol-water*, thymol dissolved in water to the extent of about 1 per cent.

The division of aniline colours into nuclear, plasmatic, and diffuse stains, is purely arbitrary, as the results of staining can be made to vary according to the different ways in which the aniline dye is used.

The following is a fairly complete list* of the most important aniline colours. It does not profess to be exhaustive:

Brown ...**Bismark**—partially sol. in water, sol. in dilute spirit.
Vesuvin—sol. in water.
Chrysoidin—sol. in water.

Red .....**Aniline scarlet**—insol. in water, freely sol. in methylated spirit.
Flamingo (deep brownish-red)—partly sol. in water, freely sol. in spirit.
Ponceau (deep red, crimson)—partly sol. in water, freely in methylated spirit.
Rosanilin—partly sol. in water, freely in dilute spirit.
Fuchsin—partly sol. in water, sol. in dilute spirit.

Orange ...**Aurin**—insol. in water, fairly sol. in absolute alcohol.
Aniline orange—insol. in water, fairly sol. in absolute alcohol.
Tropœolin (in deep-yellow glistening scales)—partly sol. in water, more so in methylated spirit.
Phosphin (yellowish-orange)—partially sol. in water, more so, but not freely, in spirit.
Safranin—sol. in water and in spirit.

Yellow ...**Fluorescin** (greenish-yellow)—insol. in water, sol. in spirit, the solution being beautifully fluorescent.
Aniline primrose—only partially sol. in spirit.

Green ...**Iodine green** (blue-green)—freely sol. in water or spirit.
Malachite green (a less blue-green)—freely sol. in water and in spirit.

* Taken from *Quarterly Journal of Microscopical Science*, vol. xxiii., p. 291, 'On Staining . . . with Aniline Dyes,' by V. D. Harris, M.D.

Blue .....**Soluble aniline blue**—freely sol. in water.
Bleu de Lyon—insol. in water, freely so in strong spirit.
Methylen blue (deep blue)—freely sol. in water and in spirit.
China blue—freely sol. in water.
Serge blue—freely sol. in water.
Blue black—freely sol. in water.

Violet ...**Hoffman's violet**—freely sol. in water and in dilute spirit.
Methyl violet (the red predominating)—partially sol. in water, freely sol. in spirit.
Gentian violet (the blue predominating)—freely sol. in water.
Tyrian blue (nearly violet)—sol. in water.
Spiller's purple—sol. in spirit, partially sol. in water.

Lee* gives Methyl Green, Bismark Brown, and Methyl Violet as the only *direct nuclear* stains; Bleu lumière, Bleu de Lyon, Indulin, Nigrosin, and Quinolein (Cyanin) as *pure plasmatic stains not affecting nuclei;* and the numerous other anilines as better ground-stains or specific stains.

According to Ehrlich the aniline colours are to be divided into two great groups, viz.: (i.) *The acid colouring matters*, comprising no less than four sub-classes, (*a*) fluorescin group, including fluorescin proper, pyrosin, eosin; (*b*) nitro-bodies—Martin's yellow, aurantia, and picric acid; (*c*) sulpho-acids, *e.g.*, tropœolin, bordeaux, ponceau, aniline black, etc.; (*d*) primary acid dyes, *e.g.*, rosolic acid, alizarin, cœrulein, etc. (ii.) *Basic dyes*, *e.g.*, fuchsine, methyl violet, methyl green, aniline blue, safranin, Bismark brown, dahlia, and gentian violet. This author further adds that the basic aniline dyes are almost the only ones which stain bacteria with any certainty; speaking roughly, the basic dyes are also the best nuclear dyes. Some of the above-named dyes are not, however, strictly speaking, anilines at all.

C.—Metallic Stains.

Silver Nitrate is used when it is required to demonstrate the endothelial cells of serous membranes. The salt is taken up by the intercellular substance when fresh, and is reduced as a black precipitate under the action of light, which maps out the cells in black lines. The fresh tissue should be placed after removal from

* Those interested in this subject cannot do better than consult 'The Microscopist's Vade Mecum,' by A. B. Lee, 2nd edit., 1890, pp. 55-76.

the body in a 0·5 or 0·25 per cent. solution for ten or fifteen minutes; it should then be washed carefully in distilled water, and exposed to the light in glycerine diluted with three times its bulk of distilled water. Silver nitrate is also used to stain nerve-fibres.

Preparation of the Solution of Silver Nitrate.—Powder 5 grms. of crystallized silver nitrate finely in a mortar, and add gradually 1000 cc. cold distilled water. After the salt has dissolved, preserve in a stoppered bottle of dark glass, or in one around which some black paper has been pasted, and keep in a dark cupboard. The use of the solid silver nitrate in bringing into view the cell-spaces of the cornea will be alluded to farther on.

Ammonium-molybdate produces a bluish-gray general stain, which acts well as a base for double-staining, A 5 per cent. solution in water should be used, and the specimens should be exposed to the light for twenty-four hours.

Gold Chloride selects and stains certain tissues, principally the nervous; it also brings out the cells of fibrous connective tissue, cartilage, and cornea.

Method of Gold Staining.—Of the many ways of using this reagent the following may be considered one of the most generally useful. Any modification necessary will be noticed in the places where it is required. The tissue is removed from the animal immediately after it has been killed, and is placed in 0·5 per cent. solution of gold chloride for half an hour to an hour; it should then be washed in distilled water, kept in a *warm*, dark place in a saturated solution of tartaric acid for three or four hours, and afterwards exposed to the light in equal parts of glycerine and water.

Immersion in filtered lemon-juice, in citric acid, or in dilute formic acid for about five to ten minutes previous to the bath of gold chloride, is advised by some. Various acids, too, are recommended instead of tartaric, as citric (saturated solution), formic (20 per cent.), acetic (4 per cent.), oxalic ($\frac{1}{2}$ per cent.). The double chloride of potassium and gold may be substituted for the gold chloride.

Method of preparing the Solution.—The gold salt is sold in sealed glass tubes, containing about 1 grm.; the tube should be broken, and the salt should be dissolved and preserved in a manner similar to that described above under the heading of Silver-nitrate Staining.

Palladium chloride, an irregular general stain, yellow to black, in solutions varying from 0·1 to 0·05 per cent., may be used; it has the same effect as gold chloride in hardening tissues, and at the same time staining them.

Osmic acid, as well as the two preceding salts, possesses the property of hardening as well as staining tissues placed in it. It is usually sold in 1 per cent. solutions, which may be diluted with distilled water at pleasure. The solution must be kept in a dark glass bottle. Osmic acid stains fat-globules black, and *brings out* the medullary sheath of nerves. Specimens to be stained with this reagent must remain in it for about an hour, and should then be removed to spirit. The special uses of this extremely valuable reagent will be noticed elsewhere.

DEHYDRATION.

Sections of tissues or organs which have been stained require to be dehydrated before they can be further prepared for mounting. The dehydrating agents are either **alcohol** in various strengths up to absolute, or, as is recommended sometimes, **aniline oil.**

CLEARING.

After dehydration, the sections have to be subjected to the action of some so-called **clearing agent.** The functions of these reagents, which are themselves liquids of a high refractive power, are, firstly, to render the tissues transparent by penetrating among the structural elements; and secondly, to take the place of the alcohol. They are capable of being in turn replaced by the mounting medium, which is generally some resinous substance, such as Canada balsam in solution.

The most useful clearing agent is **clove oil.** It is, when pure, of a very pale hue; it clears very quickly and thoroughly; it has one disadvantage—that is, that it is apt to decolorize sections stained with aniline dyes. For aniline-stained preparations, therefore, it is, perhaps, better to use **bergamot oil.** Very many other ethereal oils have been suggested as substitutes for clove oil, but without much reason it would appear. As a cheap substitute, **turpentine**, 3 parts, and **kreasote**, 1 part (mix, warm and filter), may be mentioned.

OF THE DIFFERENT METHODS OF PREPARING CUT SECTIONS FOR MICROSCOPIC EXAMINATION.

It will now be as well to epitomize briefly the method which is most suitable in each case for staining and preparing for the microscope sections of tissues cut according to the several methods described in the preceding pages. For this purpose the subject may best be treated thus:

Staining and preparing of sections which have been cut—A. **By hand after embedding in wax**, or **by the freezing microtome** with hæmatoxylin, or other such stain, with aniline dyes, etc.; B. **After saturation with paraffin**; and C. **After treatment with celloidin.**

A.—HOW TO STAIN AND PREPARE SECTIONS CUT BY HAND OR BY THE FREEZING MICROTOME.

(*a*) **With Hæmatoxylin.**—This method is recommended to the beginner as the simplest; it is of almost universal application. The sections cut by hand from pieces of material embedded in wax are, after cutting, placed in methylated spirit, in which the fragments of wax fall away from them; they may then be placed in distilled water. Sections cut with the freezing microtome should be placed in tepid water to thoroughly remove the adhering gum, and should then be replaced in cold distilled water. Unless another reagent is specially advised, as a matter of course hæmatoxylin should be employed, as it is the easiest to use, and, for the histologist, the best of all stains.

Sections of tissues, hardened in any chromium preparation, should be transferred before staining to a watch-glass containing a solution of sodium bicarbonate 1 per cent., and should be allowed to remain in it for five minutes, in order that any free acid in the hardening reagent may be neutralized. They should then be well washed in warm distilled water (30° to 40° C.).

To prepare the staining solution, a large watch-glass should be filled three-quarters full of distilled water, and from five to ten drops of aqueous or other solution of hæmatoxylin should be added. The hæmatoxylin solution should be kept in a bottle provided with a funnel and filter-paper, as *the reagent must always be filtered before it is used.*

Having made the solution, and thoroughly mixed it, the sections should be placed in it carefully one by one with a broad needle, and if they float, should be pressed down; they should be left in the fluid for some minutes. The time required varies, as some tissues stain much more quickly than others, and it is therefore necessary as a control to take out a section out of the stain from time to time, and to place it in a watch-glass full of distilled water, so that the staining may be watched and regulated. Great care is necessary, in order that the specimens be stained neither too much nor too little. The examination of the control specimen in the watch-glass should be done with a white background, which may be obtained by placing the

glass on a white filter-paper, or on a white glazed tile. Such a tile may easily be obtained, and will be found a great convenience.

It may not be out of place to notice here that stained specimens should always be examined during manipulation in this manner, and that unstained specimens may be most conveniently examined if placed in a watch-glass upon a black plate. This black plate is generally a square piece of glass with the back blackened and protected with varnish. A dark-coloured glazed tile will answer the purpose.

When the sections are sufficiently stained, they must be washed in distilled water and placed in methylated spirit, ready for the next processes of further dehydration in absolute alcohol and clearing in clove oil.

Washing in a very dilute solution of hæmatoxylin (three or four drops to a watch-glassful of water) may be substituted in place of the washing in the warm distilled water.

If the sections be accidentally overstained, they should be immersed in a 0·5 per cent. solution of hydrochloric acid for a few seconds.

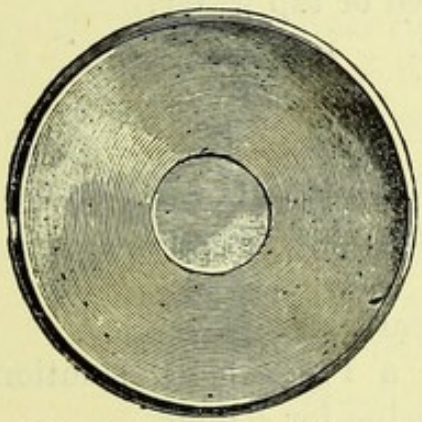

Fig. 27.—Watch-Glass.

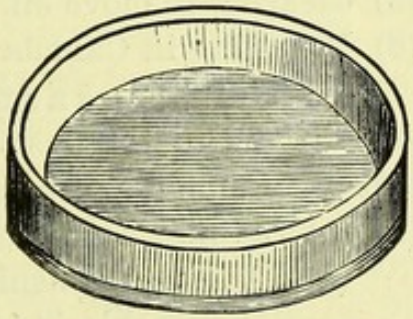

Fig. 28.—Glass Capsule.

Watch-glasses and capsules of various sizes should be obtained. The small, thin, shallow glasses are of no use; those recommended (*e.g.*, Figs. 27 and 28) are large, thick, and deep. The capsule which is used to contain spirit for hand-cutting must be large

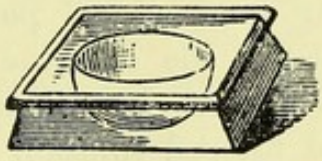

Fig. 29.—A Solid Glass Capsule.

and capacious—2½ to 3 inches in diameter, and 1½ inches deep. Instead of ordinary watch-glasses, solid glass capsules (Fig. 29) will be found very convenient.

A summary of the hæmatoxylin method is as follows:

(1) Neutralization in 1 per cent. solution of sodium bicarbonate, 3 minutes.
(2) Washing in distilled water, 2 minutes.
(3) Staining in hæmatoxylin, 2 to 5 minutes.
(4) Dehydration in methylated spirit, 3 minutes.
(5) Further dehydration in absolute alcohol, 3 minutes.
(6) Clearing in clove or bergamot oil, 3 minutes.
(7) Mounting (p. 63) in Canada balsam.

(*b*) **With Carmine.**—Staining with carmine when in an alum solution is practically the same process as with hæmatoxylin, but with lithium carmine and borax carmine the sections require a longer immersion in the dye—viz., from 10 minutes to 2 hours. The process is as follows:

(1) Staining: lithium carmine, 2 to 3 minutes; borax carmine, 5 to 15 minutes.
(2) Washing in a mixture of hydrochloric acid, 1 part, and methylated spirit 70 per cent., 100 parts, ½ to 1 minute.
(3) Thorough washing out in distilled or tap water.
(4) Dehydration in absolute alcohol.
(5) Clearing in clove oil.
(6) Mounting in Canada balsam.

(*c*) **With Picrocarmine.**—Considerable differences of opinion exist as to the best way of using this dye. Three of the methods recommended are as follows:

(i.) (1) Staining for 1 hour in picrocarmine.
(2) Thorough washing out in a 1 per cent. solution of hydrochloric acid in glycerine for ½ hour.
(3) Washing in water for 5 minutes.
(4) Washing in a yellowish-coloured spirit solution of picric acid.
(5) Clearing in clove oil.
(6) Mounting in Canada balsam.

(ii.) Woodhead's method* is as follows: 'Take of pure carmine 1 part, strong ammonia 3 parts, and distilled water 3 parts. Dissolve the carmine in a test-tube with the ammonia and water. To this add 200 parts of a cold, saturated, and filtered solution of picric acid, and mix thoroughly. Place the fluid in a basin, and cover with a bell-glass (to keep out dust), and allow it to evaporate in *strong sunlight*, testing it every few days by staining a section of skin until the nuclear and fibrous tissue are

* 'Practical Pathology,' p. 53.

stained distinctly pink, and the epithelial cells, especially those of the horny layer, are stained yellow. The best double-staining is usually given before the fluid has evaporated down to half its bulk, and at this stage it is sometimes found that crystals of picric acid are deposited in the tissues. To obviate this, it is necessary to add 10 to 20 parts of distilled water. To prevent the growth of fungi, add 2 drops of 1 to 20 carbolic acid solution to each ounce of the fluid; filter and keep in a glass-stoppered bottle. To stain a section, lay it out flat on the glass-slip, draw off the superfluous water, and run several drops of the staining fluid (not diluted) over it, allow it to stand for from 3 to 5 minutes exposed to the light, covered with a watch-glass to keep off the dust. (In winter it is well to warm gently over a spirit-lamp the slide on which the section is being stained, as slight heat causes the tissues to stain both more rapidly and more brilliantly.) *Don't wash* the section, but simply run off the superfluous fluid by tilting the slide and then wiping round the section with the thumb or a very soft clean cloth; but be careful not to remove the whole of the staining fluid, as any slight excess is gradually taken up by the tissues after the section has been mounted in either Farrant's solution or glycerine, to which 1 to 5 per cent. of formic acid has been added. (Never mount a picrocarmine-stained specimen in Canada balsam or dammar mounting fluid, or the operator will be disappointed with the results.)'

(iii.) Instead of using the ordinary solution of picrocarmine, a solution of lithium carmine 1 part to 2 parts of a saturated aqueous solution of picric acid may be employed. The method of procedure is the same in both cases, but the second solution is greatly to be preferred in some cases.

(*d*) **With Aniline Colours.**—It would be unnecessary, even if it were possible, to describe the use of each individual aniline colour. It will suffice to give examples of the methods of employing some of the most useful:

(i.) *Bismark brown* may be employed as a filtered saturated watery solution made with boiling water (3 per cent.), a 2 per cent. concentrated alcoholic solution, or a glycerine-and-water solution (1 in 4). The method of using it is as follows:

(1) Staining for 5 minutes.

(2) Thorough washing in methylated spirit.

(3) Dehydrating in absolute alcohol.
(4) Clearing in clove oil.
(5) Mounting in Canada balsam.

(ii.) *Gentian violet* may be employed either in an aqueous or spirit solution, or as an 11 per cent. concentrated alcoholic solution in aniline water (Weigert's solution).

Method :

(1) Staining for 3 to 5 minutes.
(2) Thorough washing in methylated spirit.
(3) Dehydration in absolute alcohol.
(4) Clearing in xylol or bergamot oil.
(5) Mounting in Canada balsam.

According to the same method may be employed methylen blue (1 per cent. solution), Spiller's purple (1 per cent.), methyl green, methyl violet, fuchsine (1 per cent.), and other dyes.

(iii.) *Eosin* is not, strictly speaking, an aniline, but a phenol dye. Several varieties may be obtained, *e.g.*, the ordinary orange red-coloured and erythro-eosin, which is a redder dye without orange admixture. Eosin is a somewhat diffuse stain; it has, however, the special merit of staining the coloured blood corpuscles. It may be used in a 0·1 per cent. aqueous solution, or in a concentrated alcoholic solution.

Method :

(1) Staining for 1 or 2 minutes.
(2) Washing out and dehydrating in alcohol, to which a drop or two of acetic acid has been added.
(3) Clearing in clove oil (quickly).
(4) Mounting in Canada balsam.

Eosin is chiefly employed as a double stain with some such marked nuclear stain as hæmatoxylin.

B.—HOW TO STAIN AND PREPARE FOR THE MICROSCOPE SECTIONS CUT AFTER THE PARAFFIN SATURATION METHOD.

If the sections are not to be mounted in series on the slide, they may be at once placed in slightly warm turpentine or xylol to free them from wax, and when once free from this may be washed in methylated spirit and transferred to water. After these processes they may be stained and prepared according to any method included under A; in fact, the sections are then practically the same as if they had been cut with a freezing microtome.

As a rule, however, sections cut in ribbons may be at once mounted on slides in series, and subjected to all the succeeding manipulations under those conditions.

The after-treatment is as follows: A ribbon of the sections to be mounted having been cut, a clean slide of the required size is taken and very lightly brushed with a solution of **glycerine-albumen** (made by taking egg-albumen and glycerine equal parts, shaking well up together, and adding a few drops of liquid carbolic acid to preserve it, Mayer's formula) with a camel's-hair brush previously squeezed out of water; the ribbon is then fixed with the right thumb at one end of the moistened slide, the ribbon being stretched with the left hand and brought carefully down to the slide, when in good position it is evenly pressed down to the cementing material by means of a camel's-hair brush (previously wetted and then squeezed out of the excess of water) from right to left, the left hand still holding the left end of the ribbon and keeping it taut.

When the ribbon is well arranged, the slide is exposed to a temperature of 60° to 70° C. in an oven for an hour or more. This latter process coagulates the albumen, and causes the sections to be well fixed, and at the same time melts the paraffin. After the proper time in the oven, the slides should be at once placed warm in turpentine or xylol to remove the paraffin. A method of staining the sections with Ehrlich's hæmatoxylin is thus summarized by Professor Lowne:*

(1) The slips are transferred from turpentine and placed face downwards in a bath of methylated spirit for at least 10 minutes.
(2) Methylated spirit and water, equal parts, for a few minutes.
(3) Distilled water (100 cc.), 10 per cent. solution of hydrochloric acid (1 cc.), for 5 to 15 minutes.
(4) Pour a few drops of Ehrlich's solution of hæmatoxylin over the sections and leave them covered with a glass for 1 to 2 hours.
(5) Wash in acid water.
(6) Place in a large quantity of hard tap water until of a blue colour for 1 hour or more.
(7) Methylated spirit and water.
(8) Pure methylated spirit.
(9) A few drops of absolute alcohol are then allowed to run on the sections.
(10) Clove oil.
(11) Canada balsam dissolved in xylol.

This method, which may be modified in various details—*e.g.*, the staining solution employed, which may be saffranin, gentian violet, or other dyes—appears to be the simplest and safest which can be

* 'The Blow-Fly,' 1890, p. 96.

given. It can be strongly recommended, not only for histological specimens, but for pathological materials containing micro-organisms.

C.—HOW TO STAIN AND PREPARE FOR THE MICROSCOPE SECTIONS CUT AFTER THE CELLOIDIN METHOD.

Sections cut in a celloidin mass (p. 36) may be, if deemed advisable, at once freed from the material by being placed in a mixture of equal parts of absolute alcohol and methylated ether, and may then be placed in methylated spirit and afterwards in water, and treated as ordinary sections. This is not the usual method, however. As a rule, celloidin sections are stained and dehydrated in the usual manner, but are then placed upon a slide and cleared in that position with oil of cloves or oil of bergamot, and when the excess of clearing fluid has been got rid of, are mounted in Canada balsam.

Sections cut in celloidin may be stained in any of the ways described under A, and may also be considered as very suitable for double-staining.

DOUBLE STAINING.

Double Staining.—To demonstrate the structure of some tissues satisfactorily, it is necessary to make use of two or more staining reagents. This process is called *double* or *multiple* staining, according as two or more dyes are used. The simplest form of double staining is that adopted for tissues which are first stained with silver nitrate, gold chloride, or similar reagent; and then with hæmatoxylin, carmine, or aniline.

The principle is to employ two dyes, differing in their affinities for nuclei, a diffuse stain and a nuclear stain being the happiest combination for double staining. As diffuse stains, neutral carmine, eosin, or picric acid, may be employed; and of the nuclear stains, hæmatoxylin is the most useful.

Method.—In double staining with *hæmatoxylin* and *carmine*, the following is the best method. Sections should be first stained in hæmatoxylin, then washed for twelve hours in water, and afterwards stained in carmine and thoroughly washed in water. Of the many other combinations, the following may be mentioned as good:

Hæmatoxylin and *Picrocarmine* (itself a double stain).

Hæmatoxylin and *Eosin.*

Hæmatoxylin and *Saffranin.*—In this combination the first stain with hæmatoxylin must be very light, and obtained by employing a very dilute aqueous solution of the dye.

Gentian or Methyl Violet and Eosin.
Methylen Blue and Eosin.
Methyl Green and Eosin.
Methylen Blue or Green, and Bismark Brown.
Fuchsine and Methylen Blue.
Fuchsine and Methyl Green.

It is unnecessary to do more than mention *treble* staining. If this be ever required, the combination recommended is: (1) *Picrocarmine;* (2) *Rosein;* (3) *Iodine Green.*

As regards staining with four colours, one of the authors has found that it may be done with (1) *Picrocarmine* or *Eosin;* (2) *Hæmatoxylin*; (3) *Aniline Rose;* (4) *Aniline Green.* If the tissue have been already stained in gold chloride, five stains will have been used. However, the processes are tedious, and not of any great practical value.

STAINING WITH SPECIAL OBJECTS.

(*a*) **To show the Karyokinetic Figures in Nuclei.**—For this, as well as for many other purposes, the tissues employed must be removed from the body immediately after the animal has been killed and subjected to the operation known as **fixing.** This consists of placing the tissue, cut into small pieces, in a relatively large quantity of a special fixing solution, whereby the parts of the tissue are made to retain their living structural appearance. A vast number of fixing solutions have been proposed, including all those substances before mentioned as hardening materials, or the same substances in different combinations. The fixing solutions most employed are picric acid, absolute alcohol, perchloride of mercury (1 per cent.), and osmic acid; but by far the best combination for karyokinesis is that known as:

Flemming's Chromo-osmo-acetic Acid Mixture.—The formula is as follows:

Osmic acid, 2 per cent. solution	4 parts
Chromic acid, 1 per cent. solution	15 parts
Acetic acid	1 part.

Method:

(1) Small pieces of tissue should be left in the solution 1 to 3 days.
(2) Should be washed in water 3 to 6 hours.
(3) Hardened successively in stronger and stronger spirit for 3 days.
(4) Should be cut after embedding in celloidin.

(5) Stained in a 1 per cent. watery solution of saffranin.
(6) Washed in a weakly acid mixture of absolute alcohol.
(7) Washed in pure absolute alcohol.
(8) Cleared in clove oil; and
(9) Mounted in Canada balsam.

Another Method:

(1) Harden in perchloride of mercury; then
(2) Stain in a dilute solution of the following mixture: Filtered saturated watery solution of methyl orange, 100 cc.; saturated solution of acid fuchsine, 20 cc.; methyl green, 50 cc., for from 6 to 24 hours.
(3) Wash out for a short time in almost absolute alcohol.
(4) Then in absolute alcohol.
(5) Clear in xylol and bergamot oil quickly; and
(6) Mount in Canada balsam.

(*b*) **To Stain in Mass.**—This is an exceedingly convenient practice for small objects, such as embryos, which are to be cut according to the ribbon method. By far the best staining solution for penetrating is the *Alum Cochineal* solution; but borax-carmine, hæmatoxylin (Delafield's), or Bismark brown, may be used.

Method:

(1) Harden or fix in alcohol.
(2) Wash out the alcohol in distilled water.
(3) Place in a stoppered bottle in filtered cochineal solution in a warm place for 24 hours, more or less, according to the size of the pieces.
(4) Thoroughly wash in distilled water.
(5) Place in absolute alcohol for 12 hours.
(6) Remove to xylol, cedar oil, or turpentine, for 24 hours.
(7) Embed in paraffin.
(8) Cut into ribbons of sections.

(*c*) **The Staining of Bacteria.**—It may be useful and not out of place to give some of the most-generally employed methods of staining bacteria.

(*a*) *Staining cover-glass films of dried material, e.g.*, sputum, pus, blood, or masses of micro-organisms from a cultivation. The material should be spread out in as thin a layer as possible upon the cover-glass, which should then be passed through the flame of a spirit-lamp, or of a Bunsen burner, three or four times to coagulate the albumen. It should then be placed, with the film downwards, upon some drops of the dye to be used in a watch-glass, for about four or five minutes; in some cases it is easier to place a few drops of the dye, sufficient to completely cover the material to be stained, upon the cover-

glass, and it should be allowed to remain upon it for the same time. The cover-glass should then be washed for about half a minute in dilute spirit (1 in 4); afterwards it should be again washed in distilled water, dried in the flame, and mounted in Canada balsam. This is the usual method, but in the case of sputum, suspected to contain tubercle or allied bacilli, another method has to be resorted to. The cover-glass preparation then requires to be double-stained. This method will be described on the next page.

(*b*) *Staining sections of tissue to show the micro-organisms* that they contain is done in much the same way, but, as a rule, the sections require to be double stained; by this means the bacteria may be made to assume one hue, and the containing tissues another.

For single stains the following solutions will be found most useful:

(1) *Gentian Violet* made according to this formula (**Weigert's**):

Gentian violet, 2 per cent. aqueous solution, 12 cc.
Saturated aqueous solution of aniline oil, 100 cc.

(2) *Spiller's Purple*, an aqueous solution, to which a few drops of methylated spirit have been added.

(3) *Methylen Blue*, a dilute aqueous solution very slightly alkaline in reaction.

With any of these dyes it is possible to stain satisfactorily the majority of the usual micro-organisms. For the purpose of **double staining**, it will be only necessary to give two or three methods.

Gram's Method.—Cover-glass preparations or sections of tissues are first of all stained deeply in Weigert's solution of gentian violet; they are then placed for half to two minutes in Gram's solution (iodine, 1 part; iodide of potassium, 2 parts; distilled water, 100 parts). This solution turns them to a deep green colour; they should be well washed in absolute alcohol until they are of a light-brown colour, and then double stained in a weak solution of Bismark brown, vesuvin or eosin.

Methylen Blue and Eosin Method.—A mixture of these two dyes may be used; the former in a saturated aqueous solution, and the latter in a 1 per cent. alcoholic solution. The eosin solution is added drop by drop to the methylen blue solution in a watch-glass as long as any precipitate which forms is redissolved. Sections should be stained in this mixture for about three minutes, should then be washed in alcohol, fixed in commercial benzine, cleared in a mixture of benzine and clove-oil, and mounted in Canada balsam.

To Stain Tubercle Bacilli, either in cover-glass preparations, or in sections, it is necessary to prepare two solutions, viz.: (1) Of carbol-fuchsine; and (2) methylen blue.

Carbol-fuchsine solution is made by taking fuchsine, 1 grm.; absolute phenol, 5 grms.; absolute alcohol, 10 cc.; and distilled water, 90 cc.

Carbol-methylen solution is made in the same way, only substituting methylen blue for fuchsine.

Methylen blue may also be used as a saturated aqueous solution instead of in the carbol solution.

Method:

(1) *For Cover-glass Preparations.*—A few drops of carbol-fuchsine should be placed upon the surface of the cover-glass, upon which is the properly-dried sputum. The cover-glass thus treated should then be gently warmed over a flame until the solution is quite hot (two minutes). The excess of the reagent should then be poured off, and the cover-glass should be washed for about half to one minute, or for a much less time if the colour disappears more quickly; it should then be very thoroughly washed in distilled or tap water, and the water should be carefully removed with filter-paper; a few drops of the methylen blue solution should then be placed upon the cover-glass, and allowed to remain upon it for two or three minutes. The preparation should again be washed with water, dried in the flame, and mounted in Canada balsam. The bacilli will appear as red rods upon a blue ground.

(2) *For Sections of Tissues* (the same treatment is also proper for leprosy bacilli).—Place about 10 cc. of the carbol solution in a porcelain dish and warm it over the flame to a temperature of about 60° C.; then put the sections of the tissue to be stained into the warm solution one by one with the help of a broad needle; leave them in this dye for five to ten minutes, then take them out and decolourize in a 33 per cent. solution of nitric acid, or a 25 per cent. solution of sulphuric acid (half to two minutes); then thoroughly wash in distilled water, this latter procedure will bring back part of the colour which was apparently removed by the acid; after this it will be best to place the sections to be double stained one by one upon slides, and to do the rest of the operations by adding the reagents to them in that position. The first of these further steps

consists of dropping upon the section sufficient of the methylen blue solution to cover it; in from two to three minutes, washing away the blue stain with absolute alcohol, taking care not to remove too much of the stain; then removing the excess of the alcohol with filter paper and placing upon the tissue a few drops of bergamot oil to clear the specimen, this may be removed when it has done its work either by filter paper or by washing it with a few drops of xylol; the section is now ready to be permanently mounted in Canada balsam. Specimens thus stained retain the red colour of the bacilli and the blue of the surrounding tissues for a very considerable time, provided that the acid has been thoroughly removed.

MOUNTING.

For this process are required:

(1) **Glass Slides,*** which are slips of glass 3 inches long and 1 inch broad (76 × 26 mm.), about the thickness of ordinary window-glass, with or without ground edges. They are sold by the makers of microscopic requisites at from 2d. to 6d. a dozen. It is as well to keep a good stock on hand; and, as a rule, within reasonable limits the thinnest are the best. The German or so-called Giessen pattern slides are smaller than the above-described or English form; they measure 48 × 28 mm. Large slides suitable for ribbons of sections are now supplied, viz., 87 × 37 mm.

(2) **Cover-glasses** are made of extremely thin glass; they are circular or square, $\frac{1}{2}$ to $\frac{5}{8}$ inch and upward in diameter. There are several kinds sold, usually known as ordinary, thin, and extra thin. The ordinary are quite thin enough for the student, but sometimes it is as well for him to provide himself with each kind. The ordinary cover-glass measures from ·004 to ·008 inch in thickness.

To measure Cover-glasses.—Thin glass may be placed edgewise in the stage forceps of the microscope and measured very accurately with the micrometer. Zeiss measures the thickness of cover-glasses by placing the glass in a specially-constructed slip, which projects from the side of a box, the reading being given by an indicator which moves over a divided circle on the lid. Each division of the circle

* These may be obtained of Stanley, London Bridge; Beck, Cornhill; Medland, London Bridge; Baker, Holborn; Crouch, Barbican; and of many other opticians.

represents the hundredth of a millimètre. The thickness of cover-glasses in ordinary use is 0·15 to 0·21 mm.

To cut Cover-glasses.—Thin glass may be bought in sheets, and cut into squares or circles by the operator with a diamond, but this is not advised.

To clean Slides and Cover-glasses.—For the former it is generally only necessary to wash them in soft water or weak soda-water, drying with a clean cloth, and polishing with chamois leather. A mixture of ether and alcohol, caustic potash, infusion of nut-galls or alcohol may, however, be used sometimes. Cover-glasses may be washed in strong potash or in infusion of nut-galls, or in alcohol, and should be dried and polished with a fine cambric pocket-handkerchief.

(3) **A Section-lifter**, which may be easily made by beating out flat one end of a thick copper wire, 4 or 5 inches in length when red-hot. The flattened portion should afterwards be filed at the edges, and rubbed smooth with sand-paper.

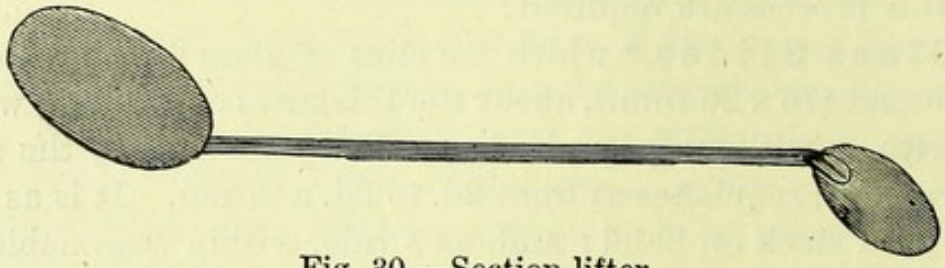

Fig. 30.—Section-lifter.

More carefully-finished section-lifters may be obtained of instrument-makers; the annexed figure represents one form of lifter suggested by the authors for mounting large sections.

MOUNTING FLUIDS.

Fresh tissues may be mounted in any of the reagents mentioned at page 22. The following fluids may also be used:

Potassium Acetate, in saturated solution, employed chiefly for mounting vegetable tissues.

Glycerine, one of the most useful of mounting fluids. It may be employed for fresh tissues, as well as for those which have been hardened and cut into sections. The fluid should be of high specific gravity; some microscopists advise dilution with a third of distilled water, but we cannot endorse this recommendation.

It must be remembered that in this reagent some tissues swell up —*i.e.*, fresh tendons—and so lose their characteristic structure, and for such the reagent is contra-indicated.

Directions for Mounting in Glycerine.—Place the tissue for a quarter of an hour in distilled water, transfer to the slide, spread out, and remove the excess of water. Then place a drop of strong glycerine on a thin cover-glass, and invert it over the specimen, taking care to keep the drop in the centre of the cover until its lowest point touches the centre of the specimen, and then allow the cover to fall gently on it. If the glycerine entirely fills the space under the cover, and is not in excess, the edges may be painted round with some cementing varnish. If too much have been taken, the excess may be removed with a capillary pipette, or with filter-paper.

Farrant's Solution is a useful substitute for glycerine, to be employed for the mounting and preserving of sections and teased specimens. It does not render the tissues so transparent as does glycerine; whilst the cover-glass becomes fixed to the slide as the solution hardens.

It is made by adding an equal weight of powdered gum arabic to a mixture of equal parts of glycerine and a saturated aqueous solution of arsenious acid.* The mixture is then allowed to stand for six weeks, being stirred at intervals. Any gum which remains undissolved is then filtered off, and the resulting clear filtrate is Farrant's solution.

Glycerine Jelly is made by taking pure gelatine 8 parts, soaking it in cold water for several hours, pouring off the water and warming the gelatine until melted, adding 1 part of egg-albumen, boiling until the albumen is coagulated and the gelatine is clear, filtering through flannel, and finally adding 6 parts of a mixture of 1 part of glycerine to 2 of camphor-water (see also Appendix). It is advisable to buy this reagent, as the making of it is difficult.

Carbolic Acid Solution (1 in 40) may sometimes be used, or a mixture of this with alcohol and arsenious acid.

Castor Oil is employed to mount crystals, etc., which are soluble in Canada balsam.

Canada Balsam.—It may be as well here to recapitulate the processes to be gone through before the sections are fit for mounting in Canada balsam. If the section be cut in spirit or water, it is passed through—

Sodii bicarb., 1 per cent.	If hardened in any preparation of chromium, to neutralize.
Distilled water.	To wash away excess of the bicarbonate.

* The arsenious acid may be omitted, and a piece of camphor may be introduced in its place.

Staining fluid.
Methylated spirit. } To dehydrate.
Absolute alcohol. }
Clove oil, or } To render transparent.
Turpentine and *Creasote*, etc. }

Preparation of Canada Balsam Solution.—The best method is to take the commercial balsam, expose it to a temperature of 70° C. for twelve hours, to dry it and render it quite hard, and then to dissolve in benzol or xylol, and filter. It should be preserved in a stoppered bottle. Ready-dried Canada balsam may now be obtained.

Another method is to mix equal parts of Canada balsam and chloroform, and warm. The balsam is entirely dissolved. Filter.

Dammar Solution.—Dissolve gum dammar in powder 50 grms. in 150 cc. turpentine, and filter; gum mastiche 50 grms. in 200 cc. chloroform, and filter. Mix the solution, and again filter. This solution can be employed in place of Canada balsam. Benzol may be employed instead of turpentine and chloroform.

Drop-bottles for Canada Balsam, Dammar, etc.—Mounting fluids such as Canada balsam are usually kept in bottles with narrow elongations of the stopper, which dip into the fluid, and will deposit it on the cover-glass in drops (Fig. 31).

METHOD OF MOUNTING.

Having placed the sections in clove oil in a watch-glass or suitable glass dish upon a white tile, as above directed (p. 50), take a clean slide and place it upon a white filter-paper. Next, by means of a section-lifter and a needle, withdraw the section from the clove oil, and bring it down upon the centre of the slide, removing the excess of clove oil with filter-paper. Then take a clean cover-glass, and place a drop of the Canada balsam solution on the centre of it, and proceed in the manner described under the head of Glycerine Mounting (p. 62), or take hold of a corner with a pair of forceps, and gradually incline it over the specimen, allowing the opposite edge to touch the slide first outside the specimen, and then allowing the other part held with the forceps to fall gently on the specimen. Either of these methods will prevent the formation of many air-bubbles under the cover-glass. It is almost impossible not to have some, but these will disappear if the specimen be kept in a fairly warm place for a few hours.

CEMENTING REAGENTS.

Sections mounted in glycerine and in similar fluids must have, and those mounted in Canada balsam or Dammar varnish may have, their cover-glasses secured with cementing material which is

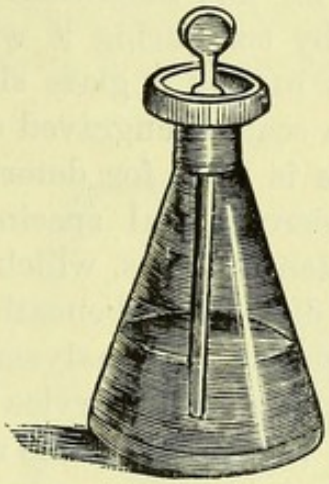

Fig. 31.—Drop-bottle for Canada Balsam, etc.

painted on with a camel's-hair brush. It would be useless to mention more than a few of these cementing fluids. The most useful are:

Dammar varnish, prepared according to the formula (p. 63).

Canada balsam, made with benzol.

Brunswick black, which is asphalte dissolved in turpentine or naphtha.

Gold-size.

Marine glue (Hollis's).—The authors find this the most convenient cement, as it dries very quickly, and is very insoluble.

ON THE MEASUREMENT OF MICROSCOPIC OBJECTS.

Microscopic objects may be measured in depth by means of the microscope itself, and in extent by a micrometer. If the thickness of the preparation is to be measured, the most superficial layer of the section must first be accurately focussed, and the position of the milled head of the fine adjustment is read off on a scale fixed to the microscope for that purpose; the fine adjustment is then altered until the lowest layer of the section is brought into focus, when the position of the milled head is again observed. In the microscopes made by Zeiss each division of the milled head corresponds to 0·01 mm. elevation or depression of the tube of the instrument in its optical axis, so that it is easy to calculate the thickness of a section.

The size of a microscopical object is measured either by the eye-

piece or by the stage micrometer. The eye-piece micrometer consists of a millimeter photographed or engraved upon a glass disc, which is placed inside the ocular in such a manner as to measure the magnified image of an object. The value of the divisions of the scale varies, however, for the particular combination of lenses which are being used, so that for any individual measurement the value must be calculated out by comparing it with a stage micrometer. The stage micrometer is merely a glass slide of the ordinary size and shape, upon which a scale is engraved or photographed.

The **micropolariscope** is used for determining the character of double refraction in microscopical specimens. It consists of a Nicol prism with a condensing lens, which acts as a Polarizer, and fits into the slide of the diaphragm beneath the stage of the microscope; and of a second prism (the Analyzer), to be placed above the ocular. The micropolariscope is of service in histology to elucidate the optical properties of voluntary muscle and to show the changes undergone during contraction.

METHODS OF INJECTING TISSUES AND ORGANS.

The distribution of the blood-vessels in any organ, of the lymph-vessels or channels, or of the bile-ducts in the liver, is demonstrated by means of injection. The material used is such that, when the operation is over, the coloured mass remains *in situ* within the vessels.

The injection of tissues is carried out either by mechanical or by physiological methods, though for ordinary histological purposes the mechanical method is alone employed.

The simplest mechanical method of injecting is by means of a syringe, and though it is the simplest and at the same time the most effectual, it requires care and great skill on the part of the operator to yield satisfactory results. This method is especially adapted for the injection of individual organs, such as the liver or kidney. The syringe used for this purpose should be short and wide in the barrel to allow of its being readily cleaned, and it should have several nozzles of varying calibre, each provided with a shoulder and a groove by which to secure it in the vessel. Instead of the pressure of a syringe worked by hand the more easily regulated pressure of a column of mercury may be used. A convenient method of utilizing this pressure is the following: The injection is put into a wide-mouthed bottle (C) provided with an india-rubber cork perforated by two holes. Into one of these holes is fitted a bent tube, which passes to the bottom of the bottle, and is in connection with the nozzle (F) by the tubing (E). A tube passes through the

second hole in the cork and brings the bottle containing the injection mass into connection with a glass receiver (B) open at both ends. The receiver B communicates by a rubber tube with a similar receiver (A) placed at a higher level than B, and filled with mercury. If the apparatus is made air-tight, it will readily be seen that by altering the height of the reservoir (A) the air-pressure in the second reservoir (B) will be modified, and consequently the pressure with

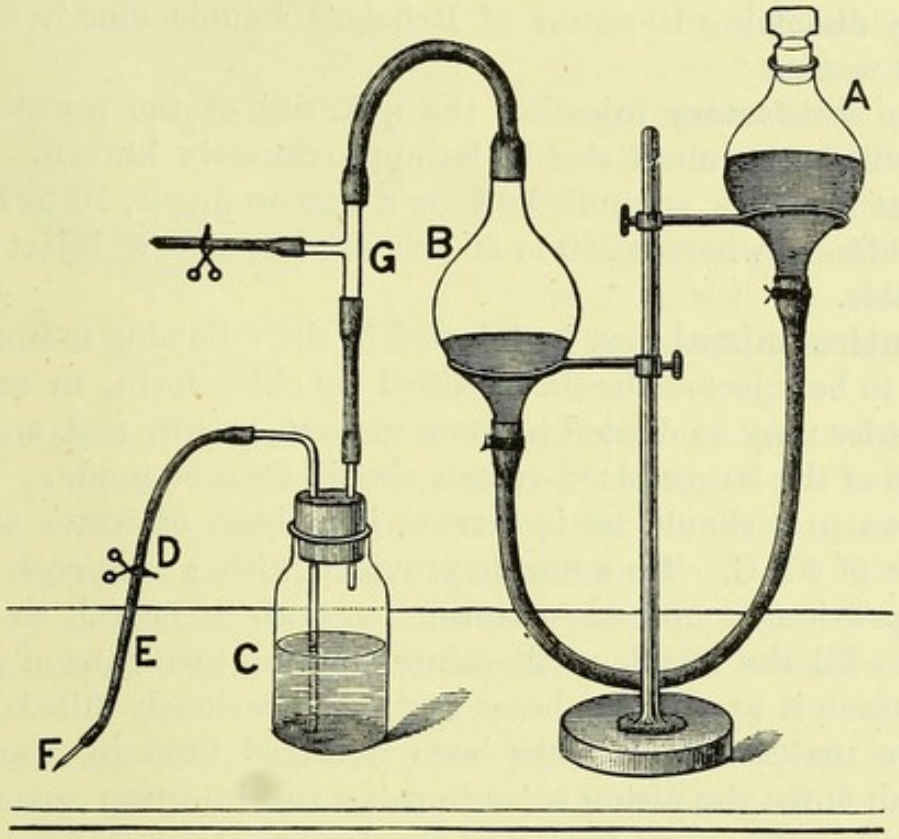

Fig. 32.—A Simple Injection Apparatus.

By raising the vessel A, filled three-quarters with mercury, the metal runs down the tube into B, which may be lowered at the same time, this compresses the air and exercises pressure on the fluid in the bottle containing the injection mass C, the cannula F is tied into the vessel of the animal to be injected, and the pressure used is gauged by a mercurial manometer, not shown in the figure, with which the T-piece is in connection at G. Stop-cocks are applied to the manometer tube, and also to the tube E at D.

which the injection mass in the bottle (C) is forced through the nozzle (F) when the clamp (D) is loosened, can at will be varied.

In carrying out an injection, the material to be injected (or the **injection mass**, as it is technically called) should first be prepared. The injection mass which is most commonly used consists of a mixture of carmine and gelatine, made as follows: Suspend 4 grams of carmine in a minimum quantity of water, adding 8 cc. of liq. ammoniæ, and afterwards 48 cc. of distilled water; filter the solution. Dissolve 13 grams of the best gelatine in 100 cc. of water by

the aid of heat derived from a water-bath, and filter. Add the carmine solution to 72 cc. of the warmed gelatine filtrate. Then add, with constant stirring, 4 or 5 cc. of glacial acetic acid to each 18 cc. of the carmine and gelatine solution, kept at a temperature of 40° C.

A solution of gelatine prepared as above, with the substitution of Berlin blue for the carmine, forms an equally good injecting fluid. The Berlin blue is an aqueous solution of a strength of 2 per cent., made by dissolving 10 grams of Brücke's soluble blue in 500 cc. of distilled water.

For a satisfactory injection the quantity of the injection mass which will be required should be approximately known. Ranvier says that 2 or 3 cc. are sufficient for a human finger, 10 to 12 cc. for a dog's kidney, whereas 250 to 300 cc. are required to inject the body of a rabbit.

An **entire animal** may be injected in the following manner: The animal to be injected should be killed by chloroform, in order that the arteries may be dilated to their utmost extent, and an incision into one of the larger blood-vessels should then be made. Whilst it is still warm it should be immersed in a bath of water at a temperature of 40° C. Tie a nozzle provided with a stop-cock into the vessel previously opened—carotid, femoral, or crural, as the case may be—fill the nozzle with saline solution by means of a pipette. Then attach it to a strong brass syringe, previously filled with the injection material, which has been rendered fluid by warming it, and push down the piston so as to drive the injection material into the vessel. This must be done very slowly indeed, and the progress of the injection should be ascertained from time to time by examining the more vascular organs of the body; *e.g.*, the tongue or ear.

Great care should be taken that no bubbles of air should find their way into the vessels, as the presence of such bubbles increases the resistance to so great an extent as to necessitate the employment of an undue amount of force. After the injection of an entire animal the body should be transferred to a large vessel containing ice-cold methylated spirit, without removing the nozzle from the artery. When isolated organs have been injected, they should be suspended in ordinary alcohol in a beaker.

The **lymphatics** can readily be demonstrated in the intestine by injecting them with a watery solution of Berlin blue. This is done by inserting the point of an ordinary hypodermic syringe, filled with the staining solution, into the coats of the intestine in the neighbourhood of a Peyer's patch, and then gently pressing down the piston.

ACCESSORY APPARATUS.

Boxes and Cabinets.—Having prepared and mounted permanent specimens of any tissue, it is necessary to *label* them carefully, noting the method of preparation, the mounting material and date, and to set them aside in a tray box, such as is seen in the figure; these may be obtained of the instrument makers. The boxes are arranged to hold from one to six dozen slides; when a larger collection has been made, the worker will find a cabinet very convenient.

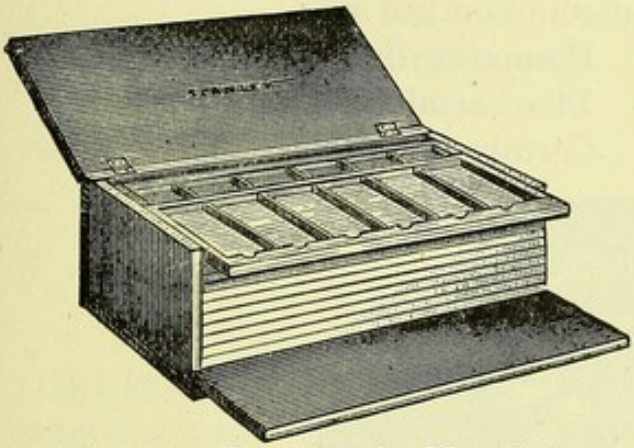

Fig. 33.—Tray Box for Mounted Specimens.

Knives.—For the various operations of dissecting tissues, cutting in pieces and scraping, ordinary scalpels are very useful. For more delicate dissections, etc., other knives are used—*e.g.*, those principally employed by ophthalmic surgeons. It is hardly necessary to add that knives must always be kept very sharp.

Scissors.—Must be fine and sharp, and of several sizes when possible.

Cabinets for microscopic mounting have been arranged by several instrument-makers containing all the requisites for the student in a compact compass. The prices vary from £1 1s. to £1 7s. 6d.

Forceps.—Should be fine-pointed and slender, if choice be allowed, but dissecting forceps are quite suitable for ordinary work.

Needles.—As before mentioned, a sewing-needle inserted into a piece of wood, leaving about $\frac{1}{3}$ or $\frac{1}{2}$ inch of the pointed end uncovered, is quite sufficient for all purposes. Broad needles are also very useful.

It may be useful now to add an epitome of the apparatus and reagents which may have to be used in microscopical work.

Apparatus:

Microscope.
A pair of scissors.
A pair of fine forceps.
Two scalpels.
Needles mounted in handles.
Section-lifters (2).
Labels.
Filter-papers.
A box or cabinet for mounted specimens.

Razor.
Glass slides and thin cover-glasses.
Watch-glasses (6).
Glass capsules.
Paraffin or white wax and olive oil mixture.
Gum solution.
Shellac and creasote.
Glycerin-abumin solution.

Reagents.—Those in most common use are:

Potassium bichromate, 1 per cent. and 2 per cent. solution.
Hæmatoxylin solutions.
Picrocarmin solution.
Carmine.

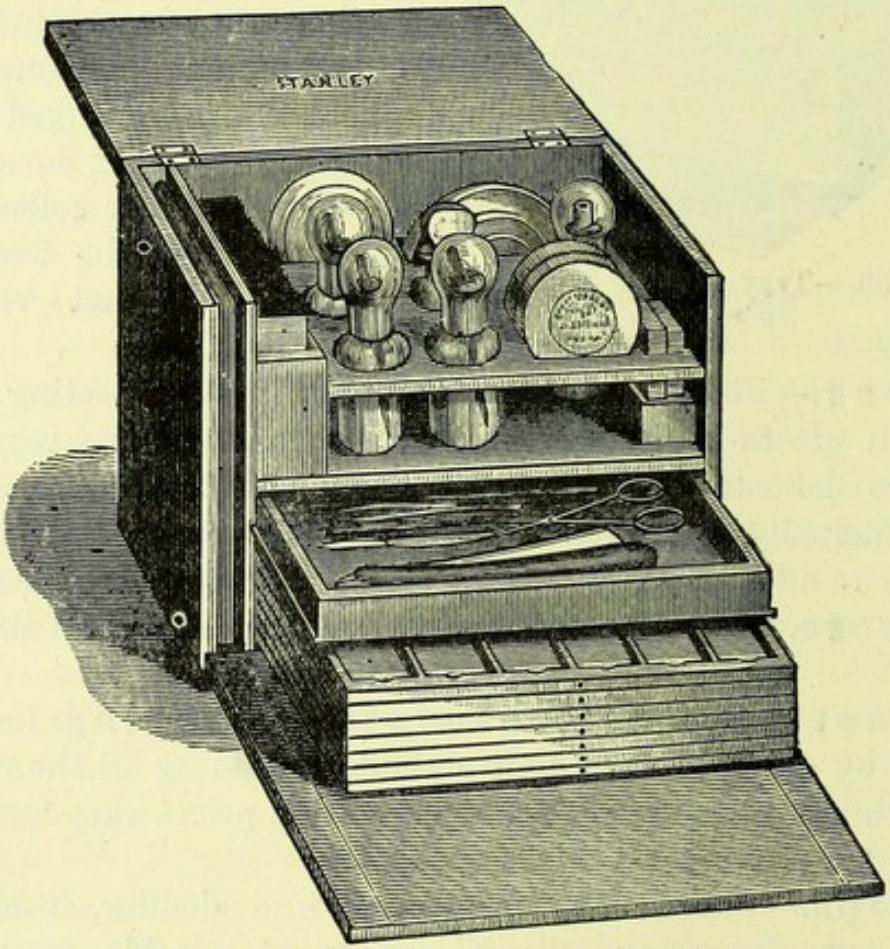

Fig. 34.—Cabinet for Microscopic Work.

Ammonium bichromate, 2 to 5 per cent. solutions.
Ammonium chromate, 5 per cent. solution.
Müller's fluid.
Chromic acid, $\frac{1}{6}$ per cent. solution.
Picric acid.
Osmic acid, gold chloride, etc.
Methylated spirit.
Absolute alcohol.
Clove, bergamot, and aniline oils, or creasote mixture.
Aniline colours.
Eosin.
Sodii bicarb., 1 per cent. solution.
Acetic, formic and tartaric acids.
Saline solution, 0·6 per cent.
Tannic and boracic acids.
Glacial acetic acid.
Sodium chloride.
Glycerine.
Dammar varnish.
Canada balsam.
Distilled water.

CHAPTER III.

THE BLOOD.

Human Blood.—Prick the finger with a needle, and apply a clean cover-glass to the drop of blood which issues, so that a little is deposited upon it; then mount quickly on a perfectly clean glass slide, taking care that the specimen does not dry up, and examine with a $\frac{1}{5}$ or $\frac{1}{6}$ objective. Notice that there are various kinds of corpuscles floating about in a fluid menstruum. These are:

(*a*) **Coloured Corpuscles.**—Circular discs about $\frac{1}{3200}$ of an inch, or ·007 to ·008 millimeter* in diameter, and $\frac{1}{12000}$ of an inch, or about ·002 millimeter, in thickness, depressed a little on each side. When seen sideways, biconcave or dumb-bell shaped; of a pale-buff colour; but when aggregated, of a reddish tint. They have a tendency to run together, collecting in rolls or **rouleaux**. Notice a corpuscle as it rolls over, and observe the change in its form, that it is alternately circular and biconcave. The corpuscle has **no nucleus**; the false appearance of a nucleus is occasioned by the refraction of light in passing through a biconcave disc. Prove this by slowly altering the focus. The centre of the corpuscle is seen to become lighter than the periphery when out of focus. The corpuscles consist of two parts: a stroma, which is colourless, and the coloured part, a red crystallizable substance, *hæmoglobin*. There is strong evidence in favour of the belief that the red-corpuscles consist of an envelope enclosing the coloured contents.

(*b*) **Colourless Corpuscles.**—Their proportion to the coloured varies from 2 to 10 in 1,000. When perfectly fresh they are spherical and faintly granular; they quickly alter,

* A thousandth part of a millimeter is called a micron, and is represented by the letter μ.

and become markedly granular, and consist of various substances, *e.g.*, lecithin, cholesterin, and cell-globulin. They are about $\frac{1}{2500}$ inch or 10μ in diameter, and contain **one or more nuclei**, not often apparent without the addition of weak acetic acid. They are nearly always isolated, and do not collect together or mix with the coloured discs. They are endowed with the power of spontaneous motion, the so-called *amæboid movement*.

(*c*) **Blood Plates.**—Notice that here and there are small more or less rounded, or slightly oval, granules, about one-third the size of the ordinary coloured corpuscles. These are the *blood plates* of Bizzozero.

Action of Reagents.—Make another preparation of a drop of blood on a slide, and add to it a drop of normal saline solution. Cover it with a cover-glass. Any reagent may now be made to act upon the blood by placing a drop of it on one side of the slip, and applying a piece of filter-paper to the opposite side. A stream of the fluid passes under the cover-glass. This is called **Irrigation.** Irrigate specimens of blood with the reagents mentioned below. The structure of the corpuscles may be thus demonstrated:

(1) *Water:* the coloured corpuscles become smooth and globular, and then disappear; their colouring matter being discharged, and a colourless stroma left. This is taken to mean that water is taken into the corpuscles by osmosis through their envelopes.

(2) *Dilute acetic acid:* the same changes take place; the colourless corpuscles swell up, become more distinct, with their nuclei more apparent.

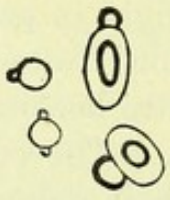

Fig. 35.—Action of Tannin upon Blood Corpuscles, those to the left are Human, and those to the right are Frogs'.

Fig. 36.—Crenate Coloured Blood Corpuscles.

(3) *Tannic acid:* the colouring matter collects in small lumps at the sides of the coloured corpuscles, and is, after a time, discharged (Fig. 35).

(4) *Saline solution*, 1 per cent., causes the coloured corpuscles to become crenate and spinous (Fig. 36), from shrivelling due to exosmosis. Syrup and other fluids of consider-

able density, and slow-drying, produce a similar appearance.

The action of other reagents may also be studied:

(5) *Carbonic acid gas:* a **gas-chamber** may be made by taking a slide and placing upon it a circle of putty; beneath the putty two small glass tubes are fixed, so that their ends extend into the cells thus formed. The blood is placed upon a cover-glass, which is inverted upon the ring of putty. One of the glass tubes is then connected by means of an india-rubber tube with the bottle in which the gas is generated. The stream of gas is allowed to pass into the chamber, and its action upon the blood corpuscles is observed. The gas passes out of the chamber through the second tube.

The preparation is brought into focus, and *carbonic acid gas* is allowed to pass through the chamber. The coloured blood corpuscles, which had become crenate from the action of the salt solution, again acquire a smooth outline, owing to the swelling up of the parts between the projections. On admitting air to the chamber the corpuscles again become crenate. The nuclei of the colourless corpuscles become more distinct. If frog's blood has been taken instead of human, the nuclei of the coloured corpuscles appear more distinct, owing to the coagulation of the substance surrounding the nucleus.

(6) *Chloroform.*—Preparation as for gases. The coloured corpuscles become globular, the hæmoglobin being finally dissolved and discharged into the plasma; the blood, when seen with the naked eye, being transparent (lake) and no longer opaque. The action of this reagent as well as that of ether is thought to show that the envelope of the corpuscles is wholly or partially dissolved by these reagents. In that case it would almost certainly contain fatty substances.

(7) *Electricity.*—The blood is placed on a slide in such a position that when it is covered it spreads between two poles of tinfoil situated 6 millimeters apart, which are connected with the secondary coil of an induction apparatus. After a succession of *induction* shocks the coloured corpuscles lose their smooth outline, become crenate, then like mulberries, and finally horse-chestnut-shaped. They swell up, and ultimately become decolourized. The colourless corpuscles swell up and dis-

integrate, their granules exhibiting molecular movements. With a *constant current* from a single Bunsen's cell the coloured corpuscles at the positive pole undergo changes which correspond to those exhibited under the action of an alkali, and at the negative pole of an acid. The colourless corpuscles assume a spheroidal form, the amœboid movements being resumed as soon as the stimulus has passed.

(8) *Alkalies.*—A mixture of 2 cc. of caustic potash in 1,000 cc. of saline solution causes both coloured and colourless blood corpuscles to swell up, burst, and disappear. The coloured corpuscles appear to be more rapidly affected than the colourless.

(9) *Alcohol.*—A mixture of $\frac{1}{3}$ spirit and $\frac{2}{3}$ water, acting upon amphibian blood, causes the nucleus to swell, and brings into view the nucleolus in the coloured corpuscles. It also renders the nuclei of the colourless corpuscles more evident, whilst one or more delicate and clear 'blebs' grow from the periphery of the colourless corpuscle. These 'blebs' appear to consist of some colloid substance, into which endosmosis rapidly takes place.

(10) *Carbolic Acid.*—With dilute solutions the coloured corpuscles shrink, and lose their regular contours; after a time they swell up, become pale, and disintegrate; the colourless corpuscles in a dilute solution of carbolic acid in saline solution (1-800 to 1-1600) do not continue their amœboid movements for so long a time or so rapidly as they otherwise would, and the movements are generally of simple extension and retraction of processes, no locomotion taking place. When the corpuscles cease to move, they do not disintegrate as rapidly as when the carbolic acid is absent. With stronger solutions the coloured corpuscles become granular, and the stroma breaks up to form a homogeneous brownish-red material of a high refractive index, which may appear either as an irregular network within the cell, or in the form of globules which tend to coalesce and are insoluble in water, but are dissolved by carbolic acid. With solutions of $\frac{1}{50}$ to $\frac{1}{20}$, the movements of the colourless corpuscles cease instantly, the cells shrink, and become coarsely granular.

(11) *Of Feeding the Colourless Corpuscles.*—The preparation of blood on the warm stage is irrigated with carmine, vermilion, or aniline blue, in a finely-divided state, or with

milk. The colourless corpuscles will be found after a short time to have ingested some of the finer particles. The particles are taken into the substance of the corpuscle by the union around it of two of the protoplasmic processes, and they thus lie at first close to the periphery of the cell, being carried at a later period nearer to its centre.

Method of Counting the Blood Corpuscles.—This operation may be accomplished by means of an instrument called the *Hæmocytometer* (Gowers).

It consists of (i.) a metal plate with a central aperture and a pair of clips; the plate is larger than (ii.) a glass slip which it holds. The slip is of the ordinary size; it is provided at its centre with a cell which is exactly one-fifth of a millimeter in depth, and whose floor is ruled in such a way as to form a series of squares, each measuring one-tenth of a millimeter (C). (iii.) A tube (B) with a bore like that of a thermometer, graduated to contain 5 cubic millimeters. (iv.) A pipette (A) of 995 cubic millimeters capacity, with a fine aperture. (v.) Elastic tubes with mouth-pieces of glass. (vi.) A small glass jar (D). (vii.) A glass stirring-rod in the form of a paddle (E). (viii.) A guarded needle (F). (ix.) Cover-glasses, wash-leather, and brushes. (x.) A bottle of sulphate of soda in solution of sp. gr. 1·025.

Fix the elastic tubes to the pipette (A) and to the finely-bored tube (B), draw up by suction 995 cmm. of the sulphate of soda solution into the former, and expel it into the glass jar. With the guarded needle, which should be perfectly clean, draw a large drop of blood from the palmar surface of the last phalanx of the left middle finger. The blood will be more easily obtained, and with less pain, if a handkerchief has been wound tightly round the finger from below upwards; the blood should be drawn by a single rapid prick of the needle. Suck up the blood into the capillary tube (B), until it extends slightly beyond the five cubic millimeters mark; remove the excess by means of a piece of clean blotting-paper applied to the end of the tube; and when exactly five cubic millimeters of blood are left, expel them into the solution of sodium sulphate which has already been measured out. Mix the blood and the sodium sulphate together by a light but rapid rotatory movement of the paddle. Place one or more drops in the cell upon the slide, cover it with a thin cover-glass, and replace it upon the tray, where it should be allowed to remain for three minutes before the examination is commenced, in order to allow the blood corpuscles to settle. The solution of blood should exactly fill the cell, neither more nor less. In cleaning the cell, it is important that it should not be rubbed, or the micrometer lines will soon be

effaced. A stream of distilled water from a wash-bottle, and the subsequent use of the soft camel's-hair brush, will be found to be effectual. Examine the blood with a high power; the corpuscles will be found lying in the squares of the micrometer. Count the number of coloured corpuscles in ten squares, putting down each as they are counted upon a piece of paper; add up the total and divide

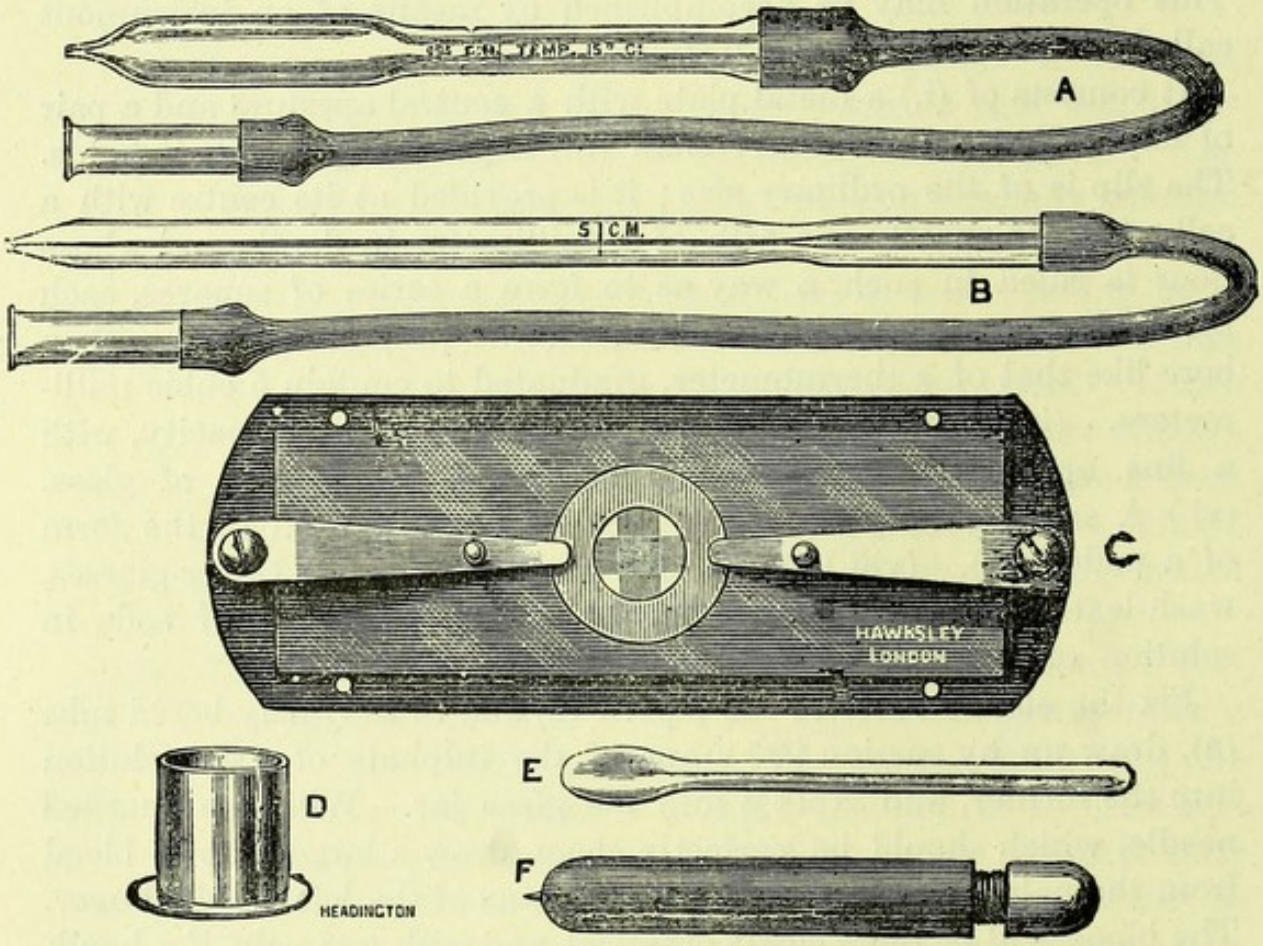

Fig. 37.—Hæmocytometer.

A. Pipette for measuring the saline solution.
B. Capillary tube for measuring the blood.
C. Cell of glass surrounding the place where the divisions are marked on the slide, mounted on a perforated metal slide, with side springs to keep down the cover-glass.
D. Jar in which the blood is diluted.
E. Mixer.
F. A guarded needle.

by ten; an average of the corpuscles for each square will thus be obtained. In the case of corpuscles which are upon the boundary lines of the squares, add or omit them according as the centre is or is not on the line bounding the square under notice at the time. In the typically healthy blood of man, each square should contain on the average fifty coloured corpuscles; in a woman the number is somewhat less. Examine the same ten squares, and count the

number of colourless corpuscles; one or two should alone be present.

Of Preparing Permanent Specimens of Stained Human Blood.—It is found in practice to be anything but easy to prepare specimens of human blood, so that the corpuscles may retain their shape and may be at the same time well stained. After the trial of a large number of different methods, the following is recommended as giving the most satisfactory results: The finger is pricked and a large drop of blood allowed to exude; a perfectly clean cover-glass is lightly drawn along the top so that a very thin layer of blood adheres, so thin as hardly to be evident until it is dry. It is then dried in the air or put at once without drying into one of the following solutions, viz.: chromic acid, $\frac{1}{12}$ per cent.; bichromate of potassium, $\frac{1}{2}$ per cent.; methylated spirit or absolute alcohol for five or ten minutes, washed in water, and again dried. The specimen is now ready for staining. The best dye for this purpose is a *recently prepared* 1 per cent. solution of Spiller's purple in water to which a few drops of alcohol have been added, or a weak spirit solution of rosein. A few drops of one or other dye having been filtered into a watch-glass, the cover-glass is placed upon the surface of the solution blood downwards, and allowed to remain so for from five to ten minutes. It is then removed, washed for some time in a gentle stream of distilled water, dried thoroughly, and mounted in Canada balsam, with or without previous treatment in clove oil for a minute or two. On examination of the specimen the coloured corpuscles should be found of normal shape and coloured purple or red, according to the dye used, whilst the colourless corpuscles are correspondingly stained. The method with Spiller's purple will be found especially useful when blood is examined in diseased conditions in which the existence of micro-organisms is suspected, and is superior to those obtained with many of the aniline dyes (such as methyl violet).

THE THOMA-ZEISS HÆMOCYTOMETER

Consists of an exactly-graduated pipette, which serves to dilute the blood to be examined to a given percentage—say 1 per cent.—and a micrometer, by means of which the blood corpuscles contained in a cubic millimeter of the diluted blood may be *counted* under the microscope.

The pipette (Fig. 38) is so graduated that the capillary stem has a capacity of $\frac{1}{100}$ of that of the ball above it. If blood be first of all drawn up to line 1, and the saline diluent be then drawn up

through the capillary stem so as to fill the ball up to line 101, we shall have 101 parts of fluid, *i.e.*, 1 part blood and 100 parts of saline solution in the pipette. As, however, the contents of the capillary tube itself are displaced unmixed, we have in the *mixture* of blood and saline solution 1 part of blood and 99 parts of solution, *i.e.*, a 1 per cent. dilution. *t* is a tube fitted with a mouthpiece (*a*), by means of which the blood taken from the tip of a finger is drawn, *e.g.*, up to line 1. After the pipette has been filled with blood and the diluent up to line 101, the two fluids in the ball are thoroughly mixed, a process which is enhanced by a small glass-pearl (*p*) fused into the ball; and while the pipette is being shaken, about half the contents of the ball are driven out by blowing into *a*. We prefer not to use the mouth at all, as it is quite impossible to prevent saliva from entering the pipette, and we find the bellows of a freezing microtome to be a very useful substitute.

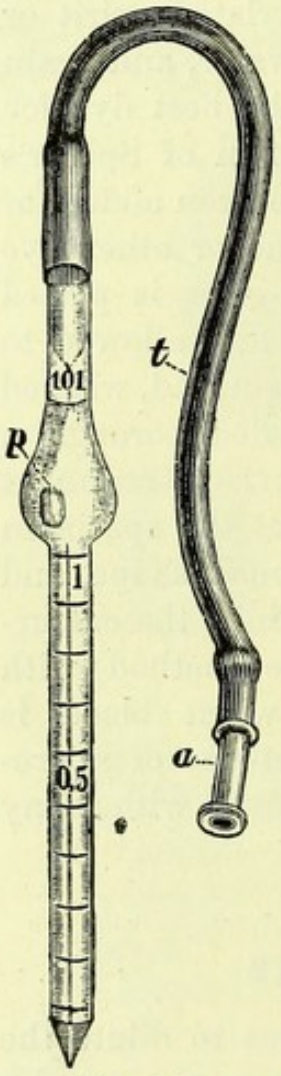

Fig. 38.

We assume that before the blood was mixed the hæmocytometer proper was put in a condition ready for use. Too much stress cannot be laid upon the imperativeness of absolute cleanliness. A short description of the instrument will make this necessity at once apparent.

The hæmocytometer consists of a glass slide (*s*) (Fig. 39), upon which is mounted a covered disc (*m*), accurately ruled, so as to present one square millimeter divided into 400 squares of $\frac{1}{20}$ mm. side. This cross-line micrometer is surrounded by an annular cell (*c*), which has such a height as to make the cell project exactly $\frac{1}{10}$ millimeter beyond *m*. If a drop of the diluted blood be now placed upon *m*, and *c* covered with the plane cover-glass, the volume of the fluid above each square of the micrometer—*i.e.*, above each $\frac{1}{400}$ millimeter—will be $\frac{1}{4000}$ cubic millimeter. If under the microscope, after counting the corpuscles in a certain number of squares visible in the field, *m* be found to be the average number of corpuscles per square of $\frac{1}{400}$ millimeter area, this number would have to be multiplied by 4000×100 to give the number of corpuscles per cubic millimeter of undiluted blood.

The slightest particle of dust or blood between the surface of *c* and the cover-glass will, it will now be easily seen, affect the dis-

tance of $\frac{1}{10}$ millimeter, and the reading will become too high. A cover-glass which is in perfect contact with the surface of the cell *c* will exhibit Newton's convexes, and this phenomenon may be used as a criterion of the accuracy of the work.

Fig. 39.

For counting white corpuscles, Zeiss recommends and supplies a melangaur, giving a dilution of 1 : 10—$\frac{1}{3}$ per cent. acetic acid being in this case a suitable diluent.

HÆMOGLOBIN CRYSTALS.

These crystals may be prepared as follows: Take a drop of blood from a guinea-pig, rat, or dog, which has been killed by ether, and let it coagulate on a slide; add a little water, then take up the clot with the forceps, and let several small drops fall from it upon another slide. Cover with a cover-glass. As these drops evaporate, hæmoglobin crystals of various sizes shoot out from the edges, separately and in bundles, varying in shape according to the animal from which the blood has been taken. In the case of the blood of the three animals mentioned, an easy way to form the crystals is to shake up some drops of their defibrinated blood in a test tube half filled with distilled water; in the course of an hour or so the sediment will be found to be chiefly composed of hæmoglobin crystals.

Copeman recommends the following method of obtaining hæmoglobin crystals from the blood of animals other than man: To defibrinated blood add one-sixteenth of its volume of ether, and shake in a stoppered bottle for some minutes until it becomes perfectly transparent or lakey. Allow the air to escape whilst it is being shaken by loosening the stopper from time to time, so that the air which was in the bottle may be replaced by ether vapour. The bottle should then be kept in a room at the ordinary temperature, and a little of the lakey blood may be removed every other day. A drop being placed upon a slide, it should be allowed to become nearly dry before it is covered with a thin cover-glass. The preparation is to be kept for an hour before it is examined, when, if the blood has stood for a sufficient length of time under the ether, radiating crystals at first small but subsequently enlarging will be seen near the edges of the cover-glass.

FLEISCHL'S HÆMOGLOBINOMETER.*

In this instrument the amount of hæmoglobin contained in blood is determined by comparing a stratum of dilute blood of given depth and dilution with a standard solid substance of a uniform tint spectroscopically similar to that of diluted blood. In order that the thickness of the standard substance may be varied, the latter is a red-tinted glass wedge tapering down to knife-edge. This 'comparison-wedge' is cemented to a colourless plane strip of glass, and is mounted in a frame (*P*), which is made to slide in a V-groove,

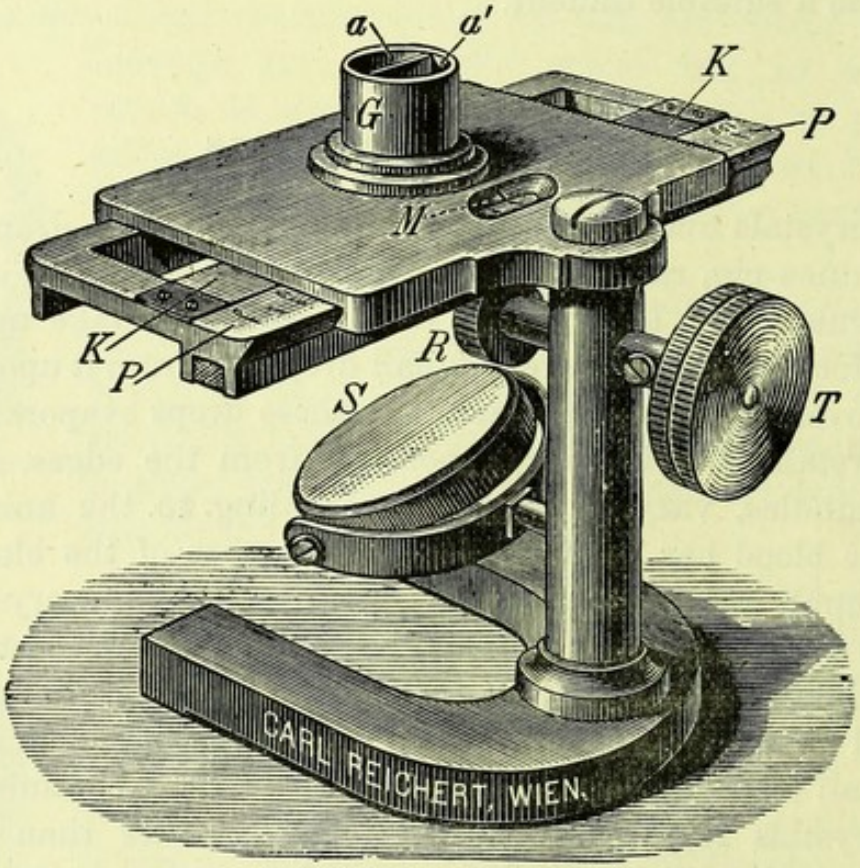

Fig. 40.—Fleischl's Hæmoglobinometer.

on the under side of the stage of the instrument. The comparison-wedge (*K*) is so placed that one of its longitudinal edges bisects the circular stage-opening, so that one-half of the stage-opening is screwed off by the red-tinted wedge. Into the stage-opening fits a small circular trough (*G*), having a glass bottom and divided into two equal compartments by a thin lamina. One compartment (*a*) being filled (in a manner to be described below) with the diluted blood under examination, the other (*a'*) with water, the

* Designed by Prof. E. von Fleischl, of Vienna; made by Carl Reichert, of Vienna; sole agent in England, R. Kanthack.

trough is so placed that the lamina is in one plane of vision with the edge of the wedge, the water-compartment (a') being above the wedge, the blood-compartment (a) above the free half of the stage-opening. By turning the milled head (T) the frame (P) with the wedge (K) may be moved backwards and forwards until a position is found where the intensity of the tints due to the stratum of dilute blood on the one hand, and to the thickness of the wedge on the other, appears to be equal.

The required degree of dilution is obtained by the use of small capillary tubes of a capacity varying from 6 to 8 or 9 cubic millimeters (the capacity of each instrument is engraved upon it). These small tubes or capillary pipettes are filled with blood, care being taken that only the contents of the tube are used, since blood-stains on the outside of the pipette would give rise to errors in the reading. The pipette is then held over the blood-compartment (a) and its contents are thoroughly washed out by small amounts of distilled water. When the compartment (a) has thus become about half-filled, the pipette is again rinsed out as completely as possible in the fluid which it contains, the water and blood being then thoroughly mixed with a small wire. Water is next dropped in through the capillary blood-pipette until the blood-compartment is exactly full, as it is essential that the surface of the fluid at the brim should be neither convex nor concave. The other compartment (a') is filled with water, in such a manner that its surface is perfectly flat and in one plane with that of the dilute blood in a. The instrument is now ready for taking the reading. The light—which should be that from a petroleum lamp or any source chiefly emitting rays from the red part of the spectrum—is reflected by the mirror (S), and illuminates both compartments (a and a'), the latter being also traversed by light which has passed through the wedge (K). By moving K laterally, by means of the milled head (T), a position of K may be found and read off at M on the scale (P) which corresponds to the apparent equality of intensity of light passing through the two strata. When taking the reading it is best to place the instrument so that the lamina between a and a' divides the area of the retina into right and left halves rather than into upper and lower halves. The upper half of the retina, which is chiefly illuminated by rays coming from the sky and objects illuminated by it, has, by continued habit, become less sensitive to light than the lower half, which receives light chiefly from the ground and objects which, owing to their position, are only moderately illuminated. This process, however, does not affect the retina with respect to 'right' and 'left,' and for this reason it is advisable to bisect the retina in manner above indicated.

AMŒBOID MOVEMENTS OF THE COLOURLESS CORPUSCLES.

In order to demonstrate the amœboid movements of the colourless corpuscles, it is necessary to make use of the *warm stage*. Of this apparatus there are various kinds. The simplest is a glass slide, to which a perforated circular plate of copper is cemented; this plate is joined to a projecting rod of the same metal. The rod communicates heat from a spirit-lamp to the plate, upon which is placed the specimen of blood to be examined. The temperature is regulated by placing a small piece of cacao butter, which melts at 35° C., upon a flattened portion of the rod near the copper disc; when the butter begins to melt the spirit-lamp should be removed.

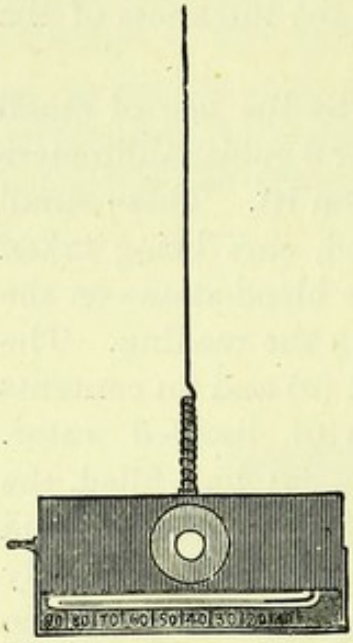

Fig. 41.—Warm Stage.

Stricker's warm stage consists of a metal-box with a central aperture. It is heated either by a copper rod, which may be fixed to it, and which may be warmed in the flame of a spirit-lamp, as in the simpler apparatus; by passing a current of hot water through the stage itself, or by means of electricity. A thermometer, whose bulb encircles the central aperture, registers the temperature. If hot water be used, an india-rubber tube should be attached to the two brass tubes which project from the sides. One of these tubes should then be placed in connection with a jug of hot water on a higher level than the stage of the microscope, whilst the other tube acts as a waste pipe. A syphon action may thus be established, and the water will circulate through the stage.

To use the warm stage, it should be placed upon the stage of the microscope, in such a position that the central aperture corresponds with the centre of the largest diaphragm, while the copper rod projects beyond the stage. A spirit lamp should be placed beneath the rod so that it is heated; watch the effect of the heat upon the thermometer, taking care that the mercury does not rise above 39° C. When it gets near this point, the spirit lamp should be moved further away. Whilst the stage is being heated, the specimen of blood to be examined should be prepared. A drop of blood drawn from the finger, or from the tail of the newt, should be diluted with an equal bulk of normal saline solution, and should be received upon a large, square, and perfectly clean cover-glass; a second cover-glass of the same size and shape should also be ready. A little olive oil or

melted paraffin should be evenly applied with a camel's-hair brush along the edges of the cover-glass holding the blood; the second cover-glass should be carefully put over it, when the blood spreads out to form a thin uniform layer; a moist chamber is thus formed by the oil preventing evaporation. The two cover-glasses containing the blood between them are then put over the aperture in the warm

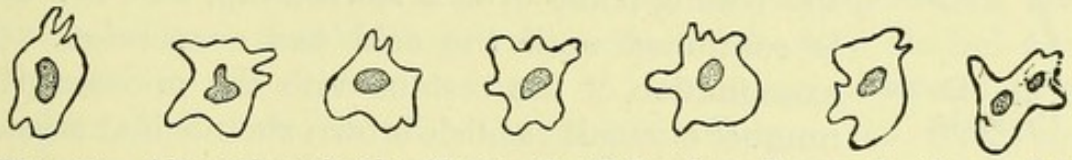

Fig. 42.—Alterations in the Shape of a Colourless Corpuscle observed for ten minutes.

stage, and examined with the highest available power, the movements of a single colourless corpuscle being noted and recorded by drawing it at intervals of a minute (Fig. 42).

Stricker's stage is expensive; but a less costly modification, which answers the purpose well, is shown in the annexed diagram (Fig. 41). It is provided with a thermometer which accurately registers slight variations of temperature. With the warm stage thus described is combined a gas chamber (see below) less rough than the simple form already described.

Gas Chamber.—In Stricker's warm stage a gas-chamber may be made by putting the tube leading from the gas generator into connection with either of the tubes which project from each side of the solid stage to which the copper rod is fixed. These tubes are on the side opposite to the thermometer; they must not be mistaken for the tubes which have been previously described as for the passage of hot water. To use the gas chamber, it is necessary to encircle the aperture with a ring of putty; the cover-glass containing the preparation to be examined is then placed upon the putty with the tissue downwards—that is to say, in the chamber. The clamp upon the gas tube is relaxed, the gas passes into the chamber, and its effect upon the preparation is noted. Air is re-introduced into the chamber by disconnecting the tubing, and sucking through or otherwise passing air into it.

HÆMIN CRYSTALS

Consist of the hydrochlorate, or hydrochloride of *hæmatin*—hæmatin is a derivative of hæmoglobin, which easily splits up into *hæmatin* and *globulin*.

The crystals of hæmin are thus prepared: A drop of blood is dried

on a glass slide. Two or three granules of common salt are added. The blood is powdered and thoroughly mixed with the salt. With a capillary pipette a drop of glacial acetic acid is added, and then the preparation is covered with a cover-glass. The blood and salt should be thoroughly dissolved in the acid. The temperature is gradually raised to the boiling-point over a spirit-lamp, and the heat is continued until the acid has evaporated. On examination of the residue with the microscope a number of small reddish-brown rhomboidal crystalline plates (Fig. 43) are seen. The specimen should be irrigated with distilled water until the excess of salt is washed away, and then dried and mounted in Canada balsam.

Fig. 43.—Hæmin Crystals.

BLOOD CORPUSCLES OF VERTEBRATES OTHER THAN MAN.

In nearly all **mammalia** the coloured corpuscles are round, disc-like, non-nucleated bodies, similar to those of man, but differing in size. In this respect they vary considerably. In **birds, reptiles, amphibia,** and **fish**, the corpuscles are oval and nucleated, the nucleus presenting a central elevation on each surface. These corpuscles are larger in birds than in mammalia; still larger in fish; and of a yet greater size in amphibia.

The general characters of the colourless corpuscles are similar in all animals, but are found in much larger proportion in the blood of fish and amphibia than in that of mammalia and birds.

The Blood of Frogs and Newts.—The blood corpuscles of the newt or frog afford an excellent means of studying on an enlarged scale, as it were, the properties of blood corpuscles in general. The coloured corpuscles of the newt are larger than those of the frog, but in each case are *oval* in shape, with a distinct *oval nucleus*, showing a fine intranuclear network; the nucleus is colourless, has a tendency to escape from the body of the cell, but bulges out the central portion of the corpuscle on each side when it is seen sideways. The colourless corpuscles vary greatly in size, the largest not being larger than the coloured corpuscle, and the smallest of the size of its nucleus. Note that some are more granular than others, and some are much more active in their amœboid movements. They possess one or more nuclei. Reagents act upon amphibian blood corpuscles much in the same way as they act upon the corpuscles of human blood.

Fig. 44.—Action of Boracic Acid on Newt's Corpuscles.

Specimens should be **irrigated** with (1) *dilute acetic acid*,

(2) *tannic acid*, (3) *boracic acid*, 1 per cent., and the effects of the reagents upon both coloured and colourless corpuscles should be watched. The last-mentioned reagent causes the colouring matter to collect around the nucleus of the coloured corpuscle, which may then be discharged from the stroma (Fig. 44).

Double-staining Blood Corpuscles.—Blood in very thin films should be allowed to dry in the air upon thin cover-glasses. These should then be placed in absolute alcohol for an hour and should again be dried. A few drops of a dilute spirit solution of fuchsin should then be poured upon the dried blood, being at once washed off, and the specimen thoroughly washed with a gentle stream of water from a wash-bottle. The cover-glass should then be dried in the flame of a spirit-lamp and allowed to cool. When ready for the second dye, a small quantity of methylen blue solution should be dropped upon it and allowed to remain for half to two minutes. A second washing with a stream of distilled water should follow until the washings are all but colourless; and the preparation should finally be dried and mounted in Canada balsam.

CHAPTER IV.

EPITHELIUM.

Methods of Examination.—Examples of the various kinds of epithelial cells may be studied either by taking scrapings from the fresh tissues, or in sections cut from the hardened material. Where the cells are arranged in layers, or *stratified*, in order to see the relation of one layer to another, the latter method must be adopted; but the shape and size of the individual cells and their nuclei may be examined according to the first method.

To prepare epithelial cells of various kinds, with their nuclei stained, it is advisable to subject the tissues from which they are to be taken to the following process: Immerse in a 2 per cent. solution of potassium bichromate for twenty-four hours after removal from the body, and then wash with distilled water until the washings are no longer yellow. Afterwards transfer to a mixture of equal parts of aqueous hæmatoxylin and glycerine for a day, and preserve in glycerine.

A. **Squamous Epithelium.**—(*a*) With a blunt knife, or with the finger-nail, scrape off a thin shred from the mucous membrane of the *cheek;* mix it with a drop of normal saline solution on a slide; place on it a cover-glass, and examine with a one-fifth objective.

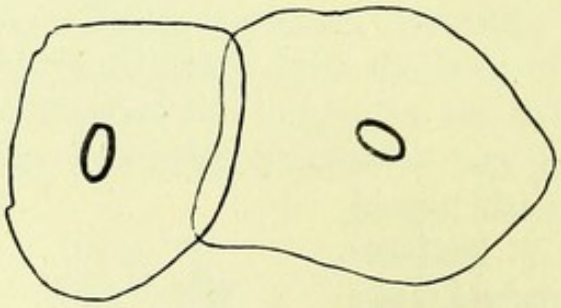

Fig. 45.—Squamous Epithelium.

The cells (Fig. 45) are large, flat, roundish, or irregularly polyhedral bodies of various sizes. Their substance is more or less transparent, containing granular matter. The nuclei are small and oval, frequently granular; they are sometimes absent, having been separated from the cells.

(*b*) Tease a scraping from the inner surface of the *œsophagus* of a cat or dog, prepared after the above method, in a small drop of

glycerine. The nuclei of the cells in such a preparation are seen to be stained purple.

B. **Columnar.**—Take a small scraping from the mucous membrane of the *intestine* of some animal (cat, rabbit, or dog),

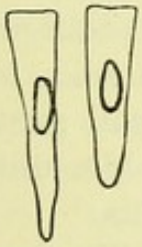

Fig. 46.—Columnar Cells.

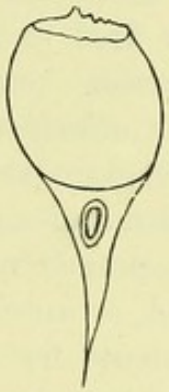

Fig. 47.—A Goblet Cell.

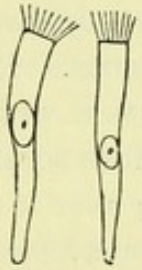

Fig. 48.—Columnar Ciliated Cells.

prepared as above, and tease it up with needles in a drop of glycerine on a glass slide.

The cells (Fig. 46) are cylindrical or conical in form, with a fairly well-defined outline; their protoplasm is finely granular; their nuclei clear, oval, and well-defined. When an aggregation of cells is seen from above, as on the surface of a villus, it has the appearance of a regular mosaic.

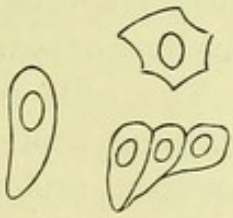

Fig. 49.—Transitional Epithelium.

C. **Transitional.**—This type of epithelium is best seen in the *bladder*, from which it may be prepared in a manner similar to the above. The shape of the cells (Fig. 49) should be noted; some are tailed, others concave on one side, spindle-shaped, or caudate. The nuclei are very large.

D. **Ciliated.**—Scrape lightly the mucous surface of a prepared *trachea*, tease out in glycerine, and examine in a similar manner.

The free border of each cell is provided with cilia (Fig. 48), whilst the deeper portion is prolonged into a fine process or tail. The nucleus is oval and well-defined.

CILIARY MOTION.

The movement of cilia may be studied by cutting off with a sharp pair of scissors, a small fragment of one of the gills (branchiæ) of a living oyster or mussel, teasing it with needles in a drop of saline solution, mounting and examining in the same fluid. The epi-

thelium scraped from the roof of a frog's mouth, or that obtained from the mucous membrane of the nose, or that lining the alimentary canal of the earthworm, is also well adapted for the demonstration of ciliary motion. The highest available magnification should be used after a general survey has been taken under a low power. Ciliary movement is seen at first to be very rapid; but it soon becomes slower, and finally ceases.

Effects of Reagents.—Dilute alkalies retard, and then stop the movements. If the cilia are working slowly, or have stopped in a preparation which has just been put up, the careful addition of a very dilute solution of caustic potash or dilute acetic acid, or the passage over it of carbonic acid, or an electric shock, will generally renew or accelerate the movements for a short time—the ultimate effect of the reagents, however, being to destroy the cilia.

Carbonic acid first accelerates, then retards, and finally stops the ciliary action, the movements recommencing if air be allowed to take the place of the carbonic acid.

Chloroform retards and finally stops ciliary action; the movements recommencing on the admission of air, if the vapour has not been applied for too long a period.

Warmth accelerates the action of cilia which were previously moving slowly, the movements ceasing at a temperature which is sufficient to destroy the vitality of the cells.

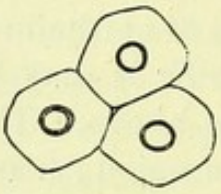

Fig. 50.—Glandular Epithelium.

E. **Glandular.**—It is advisable to postpone the detailed study of this form of epithelium until sections of glands, such as the liver or kidney, have been cut and prepared. The cells may, however, be seen by taking a scraping from the freshly-cut surface of a kidney or liver, and preparing it in salt solution (Fig. 50).

The cells vary in shape and size, are pale, and fairly well defined; their nuclei are large and round.

F. **Pigment** can be studied in scrapings from the *choroid*, *iris*, etc., teased and mounted in glycerine. The cells are either irregular and wide-branching with clear nuclei, or they are flattened and polygonal.

ENDOTHELIUM.

Endothelium is a variety of flattened epithelium which, as a single layer, lines serous membranes, blood and lymphatic vessels, etc.

Preparation.—Open the abdomen of a cat, dog, or rabbit which has been killed a few minutes previously, by bleeding, and carefully remove the *omentum* and portions of the *mesentery*, so that no hair

or blood adheres. Place them at once in a solution of silver nitrate (¼ per cent.), gently shaking out the folds in the membrane so that no part escapes the action of the reagent. Allow them to remain in the silver solution for ten minutes and then remove to a vessel of distilled water and well wash them, changing the water after the washing has been completed. Next expose them to sunlight, in the water, until they assume a brownish tint. The tissue is now ready for mounting. Small pieces should be cut off with a pair of sharp scissors, spread out upon a slide, and mounted in glycerine.

Very excellent specimens of endothelium may also be obtained from the *pleura pericardii* of the same animals, after treatment in a similar way.

In order that not only the outlines of the cells may be traced, but also that their nuclei may be evident, the tissue should be further stained in hæmatoxylin, carmine, or in one of the aniline dyes.

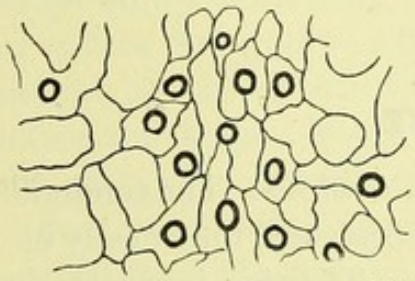

Fig. 51.—A Small Piece of the Omentum of Cat, stained in Silver Nitrate.

A good preparation of endothelium, showing numerous **stomata**, may be obtained from the frog's anterior lymph-sac (*cisterna lymphatica magna*) by filling it with the above-mentioned solution of silver nitrate by means of a capillary pipette, and, after ten minutes, removing the anterior wall, washing in distilled water, and mounting in glycerine.

On examination, the surface of the membrane is found to consist of a single layer of flattened polyhedral cells, variously modified, and forming a mosaic. The nuclei are generally single, and, except in doubly-stained specimens, appear as bright and almost colourless oval bodies within the cells. When seen in profile, they occasion projections rom the surface.

CHAPTER V.

THE CONNECTIVE TISSUES.

These comprise (1) white fibrous tissue, (2) areolar tissue, (3) elastic tissue, (4) adenoid tissue, (5) gelatinous or embryonal tissue, (6) adipose tissue, (7) cartilage, and (8) bone.

WHITE FIBROUS TISSUE.

Typical examples of this tissue are found in *tendons*. The tendons of the tail of the mouse or rat are exceedingly fine, and may easily be obtained. The tail is cut off close to its base. The skin is removed, a small piece of the extremity is pinched off between the nails, and is drawn away from the rest of the tail. In separating this piece, a number of fine threads (the tendons) will be noticed. Some of these of moderate size should be selected and teased out in saline solution.

Teasing in this manner will show the **fibrous bundles** of which the tendon is made up. Acetic acid may now be added, and the tendons will be seen to swell up and dissolve, leaving their elastic sheaths unaffected.

To demonstrate the **tendon corpuscles** several methods may be adopted:

(*a*) The first method is to place the fresh tendons in a mixture of equal parts of aqueous hæmatoxylin and glycerine for two days, teasing in glycerine, and pressing the cover-glass slightly after mounting.

(*b*) Another method is to stretch the tendons whilst still perfectly fresh upon a glass slide. The extremities of the tendons are allowed to dry, and by this means they are maintained in an extended condition. A few drops of picrocarmin are placed upon the centre, and are washed away with distilled water after the expiration of half an hour. A drop of glycerine acidified with acetic or formic

acid is then added, a hair is placed by the side of the tendon to obviate pressure, and a cover-glass is put on, the preparation being sealed up in the usual way.

(*c*) Thirdly: Good results may also be obtained by mounting the isolated tendons in a 1 per cent. solution of acetic acid, to which one-third its volume of logwood alum solution has been added. The preparation must be examined as soon as possible.

(*d*) Fourthly: By treatment with a $\frac{1}{10}$ per cent. solution of osmic acid for an hour, washing in distilled water for three hours, and subsequent staining with picrocarmin for twenty-four hours.

To demonstrate the **lymphatic spaces**, the tails of mice, after the removal of the skin, are placed in small pieces in gold chloride, left in the solution an hour, and treated in the usual manner (see p. 48) to reduce the gold. After this has been thoroughly effected they should be placed into a 2 per cent. solution of hydrochloric acid to soften the bone. From this material very thin sections should be cut, stained in hæmatoxylin, and mounted in Canada balsam.

Structure.—From preparations made in the manner above described the structure may be made out.

The tissue is seen to consist of parallel *bundles of fibres*, which vary in thickness and are held together by a homogeneous and albuminous cement substance. The individual fibres forming the bundles are straight or wavy (Fig. 52), and are extremely delicate. The acetic acid added to the tendons causes *the fibres to swell up* and to disappear, owing to the presence in the tissue of a substance which is soluble in that acid and which is readily convertible into gelatine. The bundles of fibrils are surrounded by a more or less complete sheath of elastic tissue, which is not acted upon by dilute acids; hence the constricted appearance seen in the tendons to which acetic acid has been added.

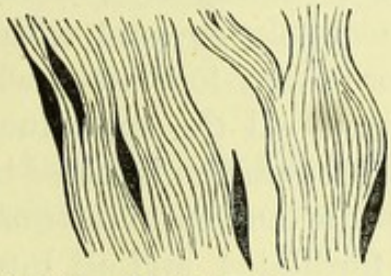

Fig. 52.—White Fibres with Connective Tissue Corpuscles.

On examination of the tendons treated to show the corpuscles, they will be found to consist of parallel bundles of fibres, whose substance is almost colourless, arranged in groups. Between every two groups is a lymph-channel, in which lie nearly parallel layers of delicately-stained cells—*the connective tissue* or *tendon cells*—forming for each channel a single continuous row of irregular angular plates (Fig. 53). Each plate is provided with a more deeply

staining nucleus. The cells are separated from each other by a cementing substance, and they possess fine processes. Each cell presents a straight ridge—*the elastic stripe*. This ridge is formed by the union of two or three concave portions of which the cell is composed, to enable it to adapt itself to the curved surfaces of the

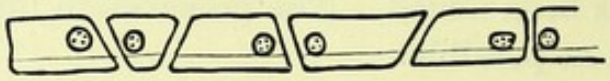

Fig. 53.—Outline Tendon Corpuscles with Elastic Stripe.

tendon bundles. *The lymphatics* are demonstrated in transverse sections of the tail, stained in chloride of gold; dark masses are seen in the tendon, corresponding to the *lymphatic channels*, filled with an albuminous fluid plasma. Radiating from these masses are fine septa—*the cement substance*—binding together the contiguous bundles.

AREOLAR TISSUE.

This form of tissue is really a variety of white fibrous tissue. By the injection into the subcutaneous tissue of a rat which has just been killed of a 0·2 per cent. solution of nitrate of silver or osmic acid, a small artificial bulla is formed. This is allowed to remain for from ten to thirty minutes, and is then opened with a pair of fine curved scissors, and the delicate subcutaneous tissue is rapidly removed and spread out on a glass slide. It is immediately covered with a cover-glass, and the preparation is stained for twenty-four hours with picrocarmin. Glycerine is passed through until all the superfluous staining material is removed, after which the preparation is sealed up.

Structure.—The tissue is composed of delicate bundles of *ordinary white fibrous tissue*, some of the fibres are fibrillated, and all inter-

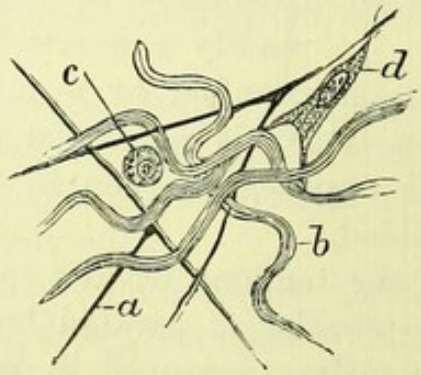

Fig. 54.—Areolar Tissue.
a, Elastic fibres; *b*, white fibres; *c*, plasma cell; *d*, connective tissue corpuscle.

lace with each other; the meshwork thus formed contains a few very fine fibrils of *elastic tissue*, which become still more evident if

dilute acetic acid have been allowed to act upon the preparation. The interspaces are filled with lymph, containing *lymph corpuscles*. Large *plate-like cells*, which appear to lie upon the surface of the bundles of fibres, are also seen. When viewed sideways, these cells have a branched appearance, and form the *plasmatic cells*. *Fat cells* are also present.

ELASTIC TISSUE.

The coarser and larger fibres may be demonstrated by teasing out in glycerine a small piece of the ligamentum nuchæ of an ox, and the finer fibres by mounting a piece of the omentum of a rabbit or cat on a slide, irrigating it freely with dilute acetic acid, and then staining with hæmatoxylin, or with an aniline dye, preferably fuchsin, and preserving it in glycerine.

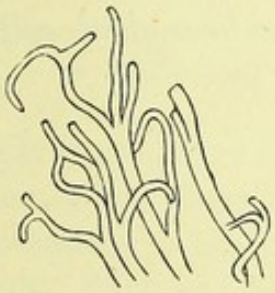

Fig. 55.—Elastic Fibres.

Structure.—On examination elastic fibres are either *thick*, well-defined, and form bundles; or they are *fine*, shining, and not in bundles. They *branch* dichotomously, and *anastomose* with each other to form a real network; when torn, they *curl up* at the ends. They do not swell up when treated with acids, and on prolonged boiling they yield elastin and not gelatine as do white fibres.

ADENOID TISSUE.

Adenoid tissue is called retiform or lymphoid tissue, and can be demonstrated by cutting and staining sections of a lymphatic gland which has been kept in dilute spirit. After staining with hæmatoxylin, the sections should be shaken in a test tube half filled with water for an hour or more. The portions of the broken-up sections should then be dehydrated, cleared, and mounted in Canada balsam.

Fig. 56.—Adenoid Reticulum, with Lymphoid Corpuscles within its Meshwork.

Adenoid tissue forms the stroma of the spleen, the lymphatic glands, the tonsils, the thymus, Peyer's glands, etc.

Structure.—It consists of a fine reticulum composed of the branchings of corpuscles, which either retain or do not retain their nuclei. In the reticular spaces are contained, closely packed together, very small corpuscles, with nuclei almost entirely filling them—the *lymphoid corpuscles*. In order to study the reticulum the corpuscles must be removed, and

this is the object of subjecting the sections to the treatment by shaking. Chemically, the fibres of the reticulum differ from both yellow elastic and white fibrous tissue.

GELATINOUS, EMBRYONAL, MUCOUS, OR WHARTON'S TISSUE.

This form of tissue is present in the umbilical cord and in the fœtal skin. From the latter it may be obtained by forming a bulla by the injection of a dilute solution of gold chloride into the subcutaneous tissue, in a stronger solution of which it is subsequently stained.

Structure.—In the youngest condition it will be found to consist of a transparent jelly-like substance, containing a hyaline mucous substance within a reticular framework. At a later period *bundles of fibrous connective tissue* are apparent, as well as *branched cells*,

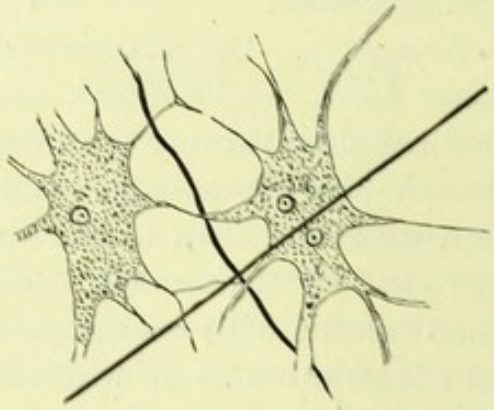

Fig. 57.—Gelatinous Tissue.

blood-vessels, and *fat-cells* in an early stage of development. The tissue yields mucin on boiling. *The vitreous humour* within the eye appears to be a variety of this tissue in which the branched cells have lost their processes.

ADIPOSE TISSUE

May be seen best in sections of scalp, or in pieces of omentum stained in hæmatoxylin. A small piece of fat, which has been partially teased, may be placed in ether for twenty-four hours, in order that the elements of the structure freed from fat may be examined. It is as well also to examine a preparation of areolar tissue formed by the injection of nitrate of silver, as well as sections of fatty tissue after they have been stained with osmic acid, in order that the action of these reagents upon the fat-cells may be observed.

Structure.—Adipose tissue consists of a matrix or network of areolar tissue containing fat-cells. *Fat-cells* are clear, well-defined, rounded vesicles of varying size, filled with an oily fluid, which

often gives rise after death to *crystalline needles*, radiating from the centre of the cell. In successful preparations, especially of the omentum, a fine zone of *protoplasm*, with a *nucleus* at one pole, can be seen surrounding the cell more or less completely. The fat-

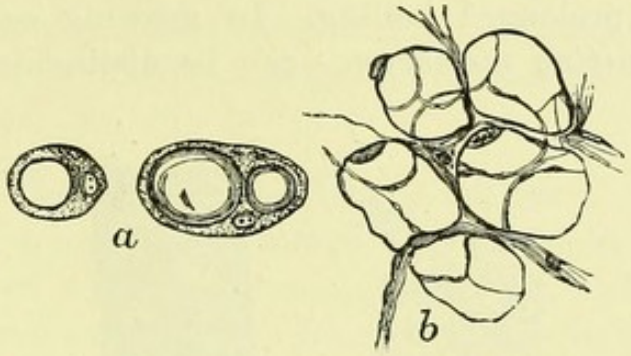

Fig. 58.—Adipose Tissue.
a, Individual fat cells; *b*, the framework and cells freed from fat.

cells may either form compact masses, with only a small amount of connective tissue, or they may be more or less isolated. The tissue possesses a capillary network of *blood-vessels*. Between the fat-cells, flattened nucleated *connective-tissue cells* may be demonstrated.

CARTILAGE.

Cartilage consists of two parts: (1) Cells; and (2) Matrix, or intercellular material. According to the nature of the matrix, cartilage is classed as:

a. *Hyaline cartilage.*
b. *Fibro-cartilage.*
c. *Elastic cartilage.*

Hyaline Cartilage.

This kind of cartilage is found in various localities, from which the names costal, tracheal articular, ossifying or intermediary, and embryonal, are derived. Portions of each of these cartilages should therefore be examined. The cartilages may be prepared in a solution of chromic acid (1 in 600), in a saturated solution of picric acid, or by the gold method. In every case the sections, which must be very thin, should be stained with carmine or hæmatoxylin. It may be as well, also, to stain some sections of the nasal cartilages in osmic acid.

Structure.—All cartilage, with the exception of the free extremity of articular cartilage, possesses a delicate vascular connective-tissue sheath—*the perichondrium*. The tissue itself consists of cells

embedded in a matrix. *The cartilage-cells* are spherical or oval protoplasmic bodies, generally containing a single nucleus. The cell protoplasm forms a fibrillar meshwork, which is contracted in embryonal and articular cartilages. Each cell is placed in a lacuna, enclosed by a firm, structureless, but transparent *matrix*, yielding chondrin on prolonged boiling. In growing cartilage, a special layer—*the limiting membrane*—can be distinguished between the

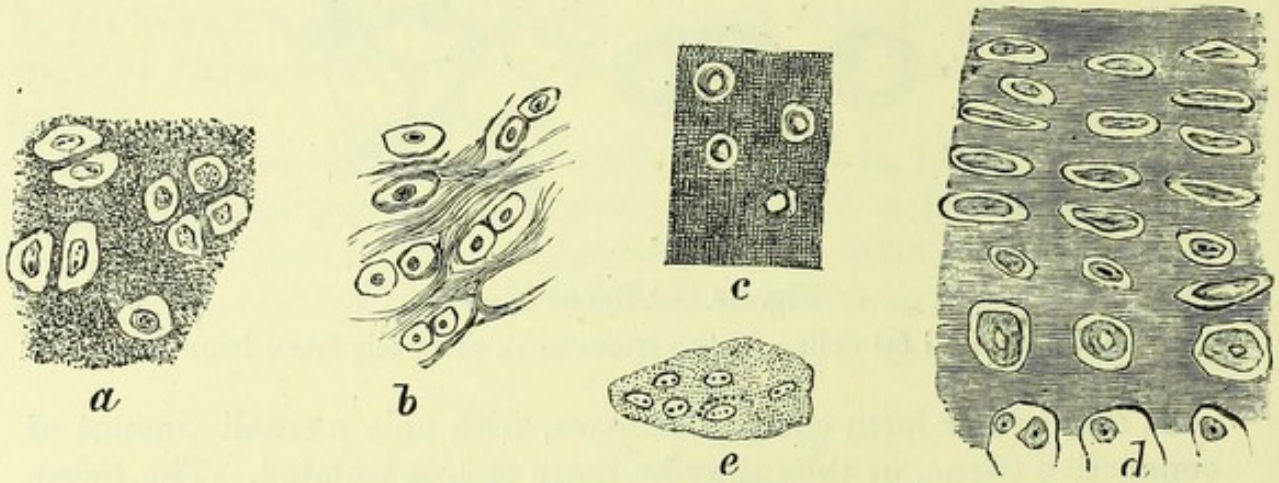

Fig. 59.—Varieties of Cartilage.

a, Hyaline; *b*, yellow elastic; *c*, white fibrous; *d*, calcifying; *e*, a multinucleated marrow-cell (osteoclast).

lacuna and the ground substance. In some cases a single lacuna may contain more than one cell, due to reproduction of the cartilage-cell by fission; and the various stages in the division of cells may often be well seen. Near the perichondrium the cartilage-cells become flattened and smaller; near the articular surface they are branched; in ossifying cartilage they are arranged in parallel rows. The matrix possesses an anastomosing system of lacunæ and canals in connection with the *lymphatic* system.

Fibro-Cartilage.

White fibro-cartilage occurs in the intervertebral substance and in sesamoid bones, and may be well seen in longitudinal sections of a mouse's tail stained in gold chloride.

Structure.—It consists (Fig. 59 *c*) of groups of slightly flattened *cells*, each with a round nucleus, and enclosed in a distinct capsule. The matrix is composed of bundles of fibrous tissue, which sometimes form lamellæ, with occasionally a concentric arrangement.

Elastic Cartilage.

This form of cartilage occurs in the lobe of the ear, in the epiglottis, in the cornicula laryngis, and in the Eustachian tube.

Sections should be cut of the pig's ear, in which it is very well developed, stained in hæmatoxylin and mounted in Canada balsam.

Structure.—Elastic cartilage (Fig. 59 *b*) in the adult is hyaline cartilage permeated by elastic fibrils. *The fibrils* are arranged so as to form the trabeculæ of a reticular framework; they branch and anastomose very frequently. The meshes contain fusiform groups of large nucleated *cells*, surrounded by a larger or smaller amount of hyaline cartilage substance.

Ossifying or Calcifying Cartilage.

In order to show ossifying cartilage, a fœtal femur or other long bone is decalcified with the chromic acid and hydrochloric acid mixture (p. 98), and sections both transverse and longitudinal of the shaft and the extremities should be cut. The sections may be doubly stained in picrocarmin (or eosin) and hæmatoxylin. This variety of cartilage is found at the junctions of cartilage with spongy bone in the epiphyses and ends of the shafts of long bones, and represents the method by which bone increases in length. It will be seen that the stages of the formation of bone in this way correspond almost exactly with the so-called development of bone in cartilage (p. 99). In a longitudinal section through the end of a growing long bone *the following layers can be seen at the junctions of the cartilage and bone:*

(1) Ordinary hyaline cartilage covered with perichondrium; in the diaphysis of a long bone at the junction of the cartilage with the spongy bone, is a characteristic arrangement of the cartilage cells in longitudinal columns. The cells are also seen to be conical in shape, pressed together and flattened transversely.

(2) A transparent layer, in which the lacunæ of the cartilage cells are seen to be enlarged, the matrix diminished, the cells enlarged and transparent, and their nuclei swollen.

(3) The lacunæ are becoming confluent, and the matrix calcified.

(4) The enlarged lacunæ are seen to be filled with marrow, and the trabeculæ of calcified cartilage are covered with layers of marrow-cells, amongst which are enlarged multinucleated mother-cells (giant-cells) (Fig. 59 *e*).

(5) The marrow-cells (osteoblasts) are seen to have deposited layers of true ossific material upon the calcified trabeculæ, and at the same time the calcified centres have become less plain.

6) The calcified centres of the trabeculæ have disappeared, leaving ossific trabeculæ, which form the spongy bone.

BONE.

The long bones of a dog, cat, or rabbit, well cleared of the surrounding tissues, should be placed for two or three weeks in a large quantity of ½ per cent. solution of chromic acid, containing 5 drops of hydrochloric or nitric acid to each ounce of the solution. When the whole of the earthy matter is dissolved out, sections should be cut with a razor in various directions, and examined in glycerine.

As the preparation of specimens of hard bone requires much time, the student is advised to buy properly prepared and mounted transverse and longitudinal sections of that material.

The structure can be best made out in a specimen of hard bone.

(*a*) **Compact Bone.**—In transverse sections of the compact tissue of long bones are seen Haversian systems (Fig. 60), more or less perfect, and Haversian interspaces. Each system consists of the central *Haversian canal* (which is generally round or oval, with an average diameter of $\frac{1}{500}$ inch, and is lined with a delicate membrane continuous with the periosteum), surrounded by concentric lamellæ of bone, between and in which are the lacunæ and canaliculi. The *Lacunæ*, $\frac{1}{2000}$ inch in length, are generally well-marked; they contain shrunken bone corpuscles. The *Canaliculi* are usually in-

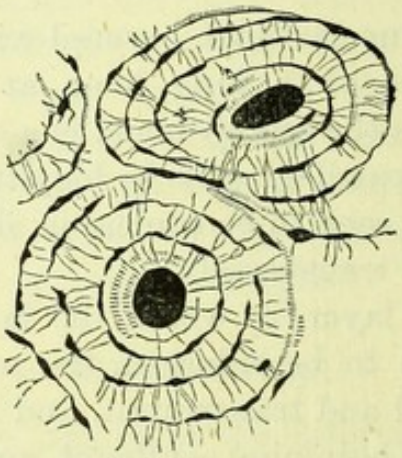

Fig. 60.—Transverse Section of Long Bone.

distinct, but they form a complete system of communication between the lacunæ, of the same and neighbouring Haversian systems and interspaces. They contain in the fresh condition prolongations from the bone corpuscles. Each Haversian system is more or less isolated from its neighbour by a layer of bone which contains but few canaliculi. The *Haversian interspaces* are the portions of bone filling up the interval between one or more of the circular Haversian systems. They do not contain any central canal; their general characters are otherwise similar to the systems.

In longitudinal sections the Haversian canals which run longitudinally are seen to anastomose freely by transverse or oblique channels. The lacunæ and canaliculi present much the same characters as in transverse sections. The Haversian canals which run near the circumference of the bone may open on the outer surface so as to admit blood-vessels from the periosteum, whilst those opening into the medullary canal receive blood-vessels, and in the case of the larger ones, medulla from the interior of the bone.

In preparations of decalcified bone it may be seen that the lamellæ are bolted together by *the perforating fibres of Sharpey*. Bone situated immediately beneath an articular cartilage differs in not possessing Haversian canals, in the lacunæ being larger than in ordinary bone, and in being destitute of canaliculi. *The periosteum* covering the free surface of bones consists of an external layer of dense fibrous tissue supplied by capillary blood-vessels, and of an internal osteogenetic layer containing a plexus of delicate connective-tissue fibrils; in the meshwork formed by these fibrils are capillary blood-vessels and a number of nucleated cells. *The medulla* is of the yellow kind, and is chiefly composed of fat-cells, with intervening membranes of flattened connective-tissue cells; it also contains numerous cells possessing one or two nuclei.

(*b*) **Spongy or Cancellous Bone.**—In spongy bones, *e.g.*, vertebral, carpal, or tarsal bones, the tissue consists of bone trabeculæ, forming a more or less open framework, in which is embedded the medullary substance. Bone trabeculæ contain lacunæ with bone corpuscles and ill-developed canaliculi. The medullary substance is of the red kind; it is rich in blood-vessels, and in cells having the characters of lymph corpuscles, fat-cells, etc.

The Development of Bone.

It is usual to describe two ways in which bone is developed, viz., (1) in cartilage, (2) in membrane; but in reality all permanent bone is developed in membrane, as will be seen in the following description. The so-called development in cartilage is really a preliminary scaffolding, as it were, and all bone which is so formed, except at the growing ends, does not remain a part of the permanent bone, but is reabsorbed nearly as soon as it is made.

In the account of the formation of bone in cartilage, the stages under which the process is described are more or less arbitrary, being inserted for the convenience of description.

In Cartilage.—The phenomena of the early stages in the formation of bone must be studied both in longitudinal and trans-

verse sections of fœtal bones which have been doubly stained in the manner above described. In Stage i. *Hyaline cartilage* is seen covered by perichondrium. *The perichondrium* consists of an outer layer of embryonal connective tissue, and an inner osteo-genetic layer containing spherical cells—the future osteoblasts and blood-vessels. In Stage ii. *The inner layer of perichondrium is seen to penetrate the cartilage*, forming for itself channels by absorption, and carrying with it blood-vessels and cells. The growth of the perichondrium inward starts at the centres of ossification. In Stage iii. *The primary marrow cavities* are formed by the appearance of lacunæ near the cartilage channels, which then become confluent, whilst the trabeculæ separating neighbouring lacunæ become calcified. The primary marrow filling the marrow cavities is the periosteal ingrowth containing the vessels and cells. In Stage iv. *The calcified trabeculæ are seen to become ensheathed with osseous material*, and then to be absorbed. A network of osseous trabeculæ instead of a network of calcified cartilage is thus formed, whilst the whole tissue resembles spongy bone (**endochondral bone**). The surfaces of the osseous trabeculæ are covered with osteoblasts, whilst the cavities separated by the trabeculæ are filled with marrow rich in vessels and cells. In Stage v. The endochondral spongy bone is seen to be absorbed from the centre outwards; the large *medullary cavity* thus being formed. Bone from the periosteum (**periosteal bone**) is also seen to be simultaneously developed round the endochondral bone, and *the osteoblasts* to multiply and to become converted into the osseous matrix and into bone corpuscles. The meshes of the spongy periosteal bone are *the Haversian spaces;* they contain marrow, by the cells of which a series of concentric lamellæ are deposited. The spaces are thus gradually reduced to *Haversian canals.* In Stage vi. *All the endochondral bone is seen to be absorbed*, and the ossified trabeculæ to be represented by the interstitial substance separating the concentric Haversian lamellæ.

As the bone increases in thickness, through the continual formation of new bone on the outside by the periosteum, the primary periosteal bone is pushed towards the centre and is absorbed, first of all becoming more spongy in its nature; the bone formed later from the periosteum being much denser and more compact in structure than that first produced.

In Membrane.—This is best studied in stained and prepared sections of fœtal lower jaw (rat or kitten). The membrane corresponds to the future periosteum; it consists of the two parts above described. In Stage i. the cells of the osteo-genetic layer—

the osteoblasts—are seen to increase and form the osseus matrix by excreting ossein around themselves, thus forming ossified trabeculæ which start from the centres of ossification. In Stage i. Portions of the trabeculæ are absorbed, whilst, as in endochondral bone, concentric lamellæ are seen to be formed by the marrow in the Haversian canals.

The formation of intramembranous bone is identical with the formation of periosteal bone. The absorption of osseous substance is in nearly every case associated with the presence of multinucleated giant cells—*the osteoclasts* (Fig. 59 *e*).

CHAPTER VI.

MUSCLE.

UNDER the microscope muscle is seen to be either *striped* or *unstriped*. There are two varieties of striped muscle, the *skeletal* (sometimes called *voluntary*) and the *cardiac*.

The following preparations should be made for the purpose of studying the minute structure of muscle:

(*a*) **Fresh Skeletal Muscle.**—

(i.) Open the chitinous shell of the leg of a water-beetle, or of a cockroach, remove a piece of the muscle to a glass slide, tease it with needles, and mount it in salt solution. Seal the edges of the cover-glass with paraffin, or Canada balsam, to prevent evaporation, and examine the preparation at once with a high power. The **transverse striation** is readily distinguished, as it is very coarse. If the preparation be examined whilst the muscle is still irritable, a wave of contraction may often be seen to pass over the fibre. Tease a piece of one of the thin muscles attached to the lower jaw of a frog, and treat it as the foregoing specimens. It will be seen that the transverse striæ are more delicate, and are therefore less readily seen than they were in the beetle's muscle.

(ii.) To demonstrate the **sarcolemma**, treat a fresh muscle with a dilute solution of acetic acid, the sarcolemma will be seen as a wavy line bounding the muscle, whilst at the same time the muscle nuclei will be more distinctly seen. If a piece of the fresh muscle of a cod be teased in salt solution, many of the fibres will be incompletely torn across, the muscle substance retracting within its sheath and exposing to view the tough, transparent sarcolemma.

(iii.) To see **Cohnheim's fields**, make sections of a fresh muscle with a freezing microtome, and examine the preparations in salt solution.

(iv.) To demonstrate the **nerve endings**, stain the flat muscles of a frog or lizard by the chloride of gold method (p. 48). The muscle should not be removed from the animal until about five hours after its death.

(v.) To demonstrate the way in which **muscle terminates in tendon**, take the sartorius with its tendinous attachments from a frog, put it into a 0·5 per cent. solution of osmic acid for several hours, and then tease it in glycerine. Macerate the sartorius of the opposite thigh in a 40 per cent. solution of caustic potash. The terminations of muscle fibres in tendon are also often well seen in longitudinal sections of the tail of a young rat or mouse which have been prepared by the chloride of gold method.

(vi.) To demonstrate the transverse muscle discs, soak a piece of fresh muscle in a 1 per cent. solution of acetic acid, or in a 0·1 per cent. solution of hydrochloric acid, and subsequently tease in glycerine.

Marshall adopts the following method of staining: The muscle fibre is immersed for a few seconds in a 1 per cent. solution of acetic acid. It is then placed for 15 minutes in

Acetic acid 1 per cent. ...	...	...	20 parts.
Gold chloride 1 per cent.	...	...	4 parts.
Osmic acid 1 per cent. ...	...	...	1 part.

After which the preparation is kept in a 1 per cent. solution of acetic acid for one or two hours in a warm chamber.

(*b*) **Hardened Muscle.**—Skeletal and heart muscle from various animals should be hardened in chromic acid and spirit; pieces should be taken and teased in glycerine, with or without previous staining in hæmatoxylin.

To see the **primitive fibrillæ**, macerate the muscles of the lower jaw of a frog, or the tail of a tadpole, in a saturated solution of picric acid for a week, or in 5 per cent. chromic acid for a few days, and tease in glycerine.

To see the general relations of muscle fibres, the position of the nuclei and the appearance of sections of muscle cut in different directions, thin sections should be made (*a*) of the tongue of a rabbit, and (*b*) of the heart of a rabbit, or guinea-pig, which should be stained in hæmatoxylin, and mounted in Canada balsam.

(*c*) **Unstriped Muscle.**—Unstriped tissue may be demonstrated by distending a piece of rabbit's intestine with saline solution, leaving it in a 1 per cent. solution of anilin black for twenty-four hours, and stripping off the outer coat with forceps. Small pieces may be mounted in glycerine.

The muscular fibre cells may also be seen by stripping off pieces of the outer coat of the intestine, which has been macerated in a solution of chromic acid, or potassium bichromate; they may be washed, stained, and teased in glycerine.

The tissue is well seen in sections of the stomach of the newt or frog which have been hardened in chromic acid and stained in hæmatoxylin; very large individual cells are well shown in the mesentery of the newt, which has been put fresh into ammonium bichromate 5 per cent. for twenty-four hours, stained in hæmatoxylin and mounted in glycerine.

With the aid of the preparations indicated above, the general *structure* of the muscular tissue can be made out. (1) It will be as well to consider, first of all, the *striped muscle* of the *voluntary* or skeletal type. It consists of long *fibres*, which are cylindrical, but appear in transverse section as rounded polygons. Each fibre is made up of a number of exceedingly fine and delicate filaments, *the fibrillæ*, enclosed within the sarcolemma. The fibres are aggregated into *bundles;* several bundles forming *fasciculi*, and these the anatomical *muscle*. The *perimysium* or fibrous connective-tissue surrounds the bundles; from it pass off between the muscle fibres small processes of connective-tissue, with cell plates and plasma cells—*the endomysium*.

So far, with the exception of the fibres and of the endomysium, the appearances may be made out with the naked eye, or with a very low power of the microscope. But now the higher powers are required.

Each fibre will be seen to consist of broad dim bands of highly refractive substance, representing the contractile portion of the muscle-fibre—*the contractile discs*—alternating with narrow bright bands of a less refractive substance—the *interstitial discs*. After hardening, each contractile disc becomes longitudinally striated, the thin oblong rods thus formed being the *sarcous elements* of Bowman. The sarcous elements are not the optical units, since each consists of minute doubly-refracting elements—*the disdiaclasts* of Brücke. When seen in transverse section, a muscular fibre appears to be subdivided by clear lines into polygonal areas—*Cohnheim's fields*, each corresponding to several sarcous-element prisms. The clear lines are due to a transparent interstitial fluid substance pressed out of the sarcous elements when they coagulate. *The sarcolemma* is a

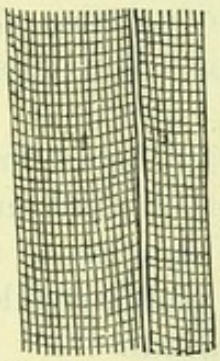

Fig. 61.—Striated Muscle.

transparent structureless elastic sheath of great resistance, which surrounds each fibre. From the sarcolemma, transverse membranous septa—*the membranes of Krause*—extend inwards across the muscle at regular intervals. By these septa the muscle-fibre is divided into equal-sized *muscle compartments*, each containing one contractile disc. The membranes of Krause are so placed that each passes across the middle of an interstitial disc, which is thus divided into two *lateral discs*.

A thin transverse median disc—*the disc of Hensen*—is occasionally seen to divide the contractile disc. In some fibres, chiefly those of insects, each lateral disc contains a row of bright granules forming, *the granular layer of* Flögel. The fibres contain nuclei, which are round, ovoid, or spindle-shaped in different animals. These nuclei are situated close to the sarcolemma, their long axes being parallel to the fibres which contain them. Each nucleus is composed of a uniform network of fibrils, and is embedded in a thin and more or less branched film of protoplasm. The nucleus and protoplasm together form the muscle cell, or *muscle corpuscle of Max Schultze*.

According to the more recent observations of Retzius, Melland, Marshall and others, each striped muscle fibre consists of a highly modified intra-cellular network homologous with the networks of other cells. This network consists of (i.) longitudinal fibrils apparently running the whole length of the fibre, and connected together at regular intervals by (ii.) the transverse networks which correspond in position to 'Krause's membranes.' Each Krause's membrane, according to this view, is therefore an open meshwork of fibrils, and not a true membrane. Each transverse network, when seen in the ordinary position of the fibre appears as a row of granules, the granules being formed by the points of junction of the longitudinal fibrils with the transverse networks. The meshes of the network are filled with semi-fluid material—the sarcous substance.

The transverse networks are connected with the muscle corpuscles, and in some cases can be traced into connection with the intracellular networks (Retzius, Marshall). Moreover, the network appears to be connected with the nerve-endings, but this is very difficult to demonstrate (Marshall).

The dark and light bands of striped muscle are due to the optical effects caused by the rows of granules. Each granule appears at low focus as a dark spot surrounded by a bright halo; the coalescence of these haloes produces a bright band crenated at each border with the row of dark spots in its centre, in the position of

Krause's membrane. The portions of the fibres between the successive Krause's membranes appear as dark bands.

The effect known as the 'Transposition of the bands' is produced in the same way, and is as follows: At low focus the appearance described above is seen, while at high focus each granule appears as a bright spot with a dark halo: hence a bright line is seen in the position occupied by the rows of black dots seen at a low focus, on each side of the bright line being a dark halo. The dark band seen at low focus remains about the same, but appears light by contrast with the dark haloes of the granules, the whole effect being an optical phenomenon caused by the refraction of light from a series of spherical bodies (the granules) immersed in a medium of lower refracting index than themselves (the sarcous substance). The network is isotropous, the sarcous substance anisotropous.

'Cohnheim's areas' are caused by the coagulation of the sarcous substance in the meshes of the transverse networks seen in transverse sections of the fibres. They are simply post-mortem phenomena.

Bowman's sarcous elements are formed in the same way by the splitting of the coagulated sarcous substance (after treatment with alcohol and other reagents) both longitudinally, along the lines of the longitudinal fibrils, and transversely, along the transverse networks.

In injected specimens it will be seen that the arteries and veins are in the perimysium; that the capillaries are in the endomysium, between the fibrillæ; and that the capillary plexus is elongated.

The individual capillaries run parallel to the muscle fibres, and anastomose with each other by short branches.

If a muscle fibre be traced to its termination in a tendon, it will be seen that the muscle substance ends abruptly in a conical extremity, whilst the sarcolemma is continued onwards as a fine thread which blends with the fibrous tissue forming the tendon. In other cases the whole muscle fibre passes into a bundle of fibrous connective tissue, with which it becomes continuous.

Heart Muscle.—A section of heart muscle shows that this variety of muscle consists of fibres which are aggregated into oblong branching masses, each with a nucleus in its centre. The individual fibres are very small, and are transversely striated, though the striæ are so delicate as only to be visible when the preparation is carefully examined with a high power of the microscope. The fibres are devoid of a sarcolemma. The oblong masses are united by a cementing substance.

Unstriped Muscle is made up of bundles of cells, bound

together by an albuminous cementing substance—*the endomysium*—in which lie connective-tissue corpuscles and a few fibres. *The perimysium*, continuous with the endomysium, is the fibrous connective-tissue surrounding and separating the bundles of muscle cells.

Fibres, fusiform, band-like, or spindle-shaped, containing elongated or staff-shaped nuclei, are placed midway in the fibres. The ends may be split into two or more parts, as may frequently be

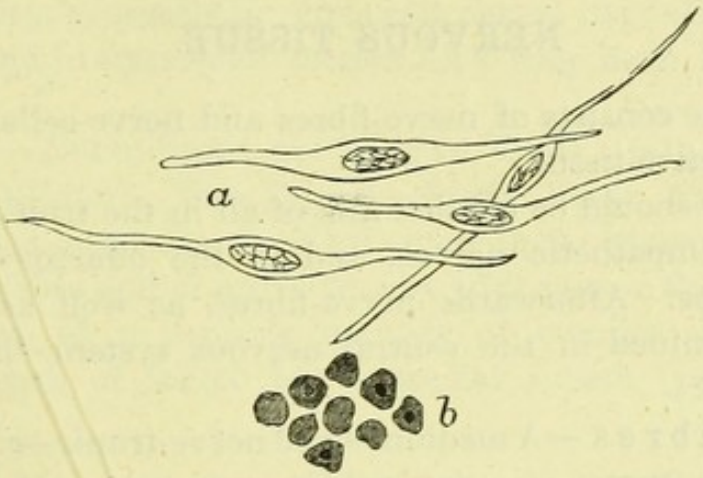

Fig. 62.—Unstriped Muscle Fibres.
a, Individual fibres isolated; *b*, transverse section.

seen in the cells obtained from arteries and veins. Each muscle cell consists of a fine sheath, probably elastic, of a central bundle of fibrils representing the contractile substance, and of an oblong *nucleus*, which includes within a membrane a fine network, anastomosing at the poles of the nucleus with the contractile fibrils. The ends of the fibres, which are usually single, may sometimes be divided. There is no sarcolemma.

CHAPTER VII.

NERVOUS TISSUE.

Nervous tissue consists of nerve-fibres and nerve-cells, with a supporting connective-tissue.

Nerve-fibres should be studied first of all in the trunks of cerebro-spinal and sympathetic nerves, and in the olfactory, optic, and auditory nerves. Afterwards nerve-fibres, as well as nerve-cells, should be examined in the central nervous system—in the spinal cord and brain.

Nerve-Fibres.—A medium-sized nerve-trunk—*e.g.*, the sciatic nerve of a cat, rabbit, guinea-pig, or frog—should be removed from the recently-killed animal, and cut into short lengths. One or more of these lengths should then be placed in the following solutions: (*a*) *Osmic acid*, 1 per cent.; (*b*) *gold chloride*, 0·5 per cent.; (*c*) *silver nitrate*, 0·25 per cent.; (*d*) *hæmatoxylin* or *carmine;* and teased with needles whilst in the reagent in order that it should penetrate properly.

Fig. 63.—A Medullated Nerve-Fibre.
a, axis cylinder; *b*, myelin or medullary sheath; *c*, external nerve-sheath; *e*, node of Ranvier; *f*, a nerve-nucleus.

The osmic acid should be allowed to act for at least an hour; and the fibres, when teased, will be found to show the medullary sheath, the nodes of Ranvier, and the sections of Lantermann, the first being stained black.

The gold chloride method will demonstrate the external nerve-sheath and the axis cylinders.

The silver nitrate requires about a quarter of an hour for its action; it will show the endothelium covering the nerve-bundles, and also, by more prolonged action, the nodes of Ranvier.

The hæmatoxylin and carmine will show, in a good preparation, the nucleated external nerve-sheath and the axis cylinder. A considerable time is necessary, however, to accomplish this action — twenty-four

hours or more—and very frequently the stain will not penetrate to the axis-cylinders even then, unless the fibres have been placed first of all in absolute alcohol, ether, or chloroform. The best form of stain to use is Delafield's hæmatoxylin (p. 43).

The fibres should in all cases be teased and mounted in glycerine.

Nerve-trunks of a rather larger size should be pinned out straight on a piece of cork, and hardened in chromic acid and spirit solution. Of these transverse sections should be cut, stained in hæmatoxylin, prepared and mounted in Canada balsam.

The auditory, sympathetic, and optic nerves may also be examined by teasing, and in transverse section after they have been hardened and stained.

Structure.—Nerve-fibres are of two kinds: (*a*) *Medullated* or (*b*) *non-medullated*. The former chiefly make up the cerebro-spinal, and the latter the so-called sympathetic system.

(*a*) The cerebro-spinal nerve-trunks, with two or three exceptions, are composed of a variable number of bundles of nerve-fibres (*funiculi*), each of which has a special sheath (*perineurium* or

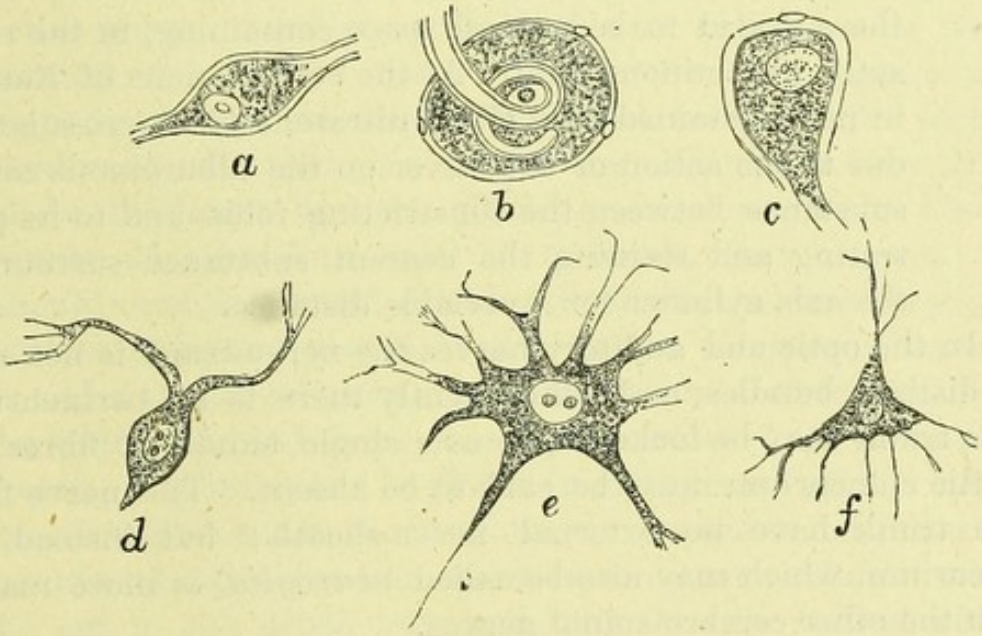

Fig. 64.—Varieties of Nerve-Cells.

a, Bipolar nerve-cell; *b*, from spinal ganglion; *c*, from sympathetic ganglion; *d*, from cerebellum; *e*, from spinal cord; *f*, from cerebral cortex.

neurilemma). These bundles are enclosed in a firm fibrous sheath (*epineurium*), which also sends in processes of connective-tissue, supporting and connecting the bundles together. Within the funiculi, between the fibres, is a delicate supporting tissue (the *endoneurium*). Each medullated nerve-fibre is made up of the following parts: (1) Primitive nerve-sheath, or nucleated sheath of

Schwann; (2) medullary or myelin sheath, or white substance of Schwann; (3) axis cylinder, primitive band, axis band, or axial fibres.

(1) *The nucleated sheath of Schwann* is the external layer of the fibre. It appears to be a simple, transparent, colourless, homogeneous structure, containing a varied number of oval nuclei embedded in protoplasm.

(2) *The medullary or myelin sheath* is the intermediate layer which forms the greater part of the nerve-tube. It surrounds the axis cylinder, and has a double contour; but at regular intervals there are constrictions in it called the *constrictions of Ranvier*. In it may be demonstrated, especially by the action of osmic acid, the *sections of Lantermann*, of which its structure is said to be made up.

(3) *The axis cylinder* is situated in the centre of the nerve-tube, and appears as a faintly-marked band, with an exceedingly fine and even outline. It is made up in the prepared specimen of a number of most delicate fibrils (*primitive fibrils*).

Between the axis cylinder and the medullary sheath there is said to be a small space containing, in the recent state, albuminous fluid. At the constrictions of Ranvier, in nerves stained with silver nitrate, a black cross is seen, due to the action of the silver on the albuminous cement substance between the constricting folds, and to its penetrating and staining the cement substance surrounding the axis cylinder for a variable distance.

(*b*) In the optic and auditory nerves the nerve-trunk is not made up of distinct bundles, and consequently there is no perineurium; but the trunk may be looked upon as a single bundle of fibres, and if so, the epineurium must be said to be absent. The nerve-fibres in the trunk have no external nerve-sheath; but instead, the endoneurium, which may also be called *neuroglia*, is more marked than in the other cerebro-spinal nerves.

(*c*) In the sympathetic system, as well as in the olfactory nerve, the nerve-fibres possess no medullary sheath, and so are called non-medullated nerve-fibres.

Spinal Cord or Medulla Spinalis.—*Methods of Preparation.*—Short lengths (about $\frac{1}{8}$ to $\frac{1}{4}$ inch) of the spinal cord of the cervical, dorsal, and lumbar regions of a calf, sheep, or pig, should be hardened in a 5 per cent. solution of bichromate of ammonium, or in the usual mixture of chromic acid and spirit, for a week, and should then be transferred to methylated spirit.

Solutions of erythro-eosin, and hæmatoxylin are recommended

for staining the sections; but carmine or picrocarmine may be used in place of the first-named dye. Stephens' blue-black ink diluted one-half or two-thirds, or anilin black, 1 per cent. solution, stains the ganglion cells excellently.

In order to study the areas or tracts in the spinal cord it is necessary to obtain pathological material from the human subject in which tracts of degeneration may be demonstrated.

The special methods which may be adopted for staining the spinal cord in order to bring out these tracts of degeneration are as follows:

(1) *Weigert's Method.*
(2) *Pal's Method.*
(3) *Pal-Exner Method.*

(1) **Weigert's Method.**—By the use of hæmatoxylin in a certain way, the dye forms what has been called a chromium or copper 'lake' with the chromium or copper salt employed in the process of hardening, staining in this way the medullary or myelin sheath of the nerves a very beautiful purple-black, whilst the connective-tissue, ganglion cells, and axis cylinders, as well as degenerated or sclerosed tissues, are coloured an orange-brown. The steps in the process may be thus summarized. The segments of spinal cord, or other portions of the central nervous system, should be hardened first of all in a solution (2 per cent.) of bichromate of potassium until of a brown (not a green) tint; they should then be infiltrated with celloidin in the usual way (p. 37), mounted on cork, and placed in a saturated solution of acetate of copper and water equal parts; or instead of the acetate, the sulphate may be employed, and kept at a temperature of 40° C. until well penetrated; they may then be placed in methylated spirit. Sections may be afterwards cut in the usual way. Instead of cutting sections of the materials after due immersion in the copper solution, sections may be cut in the usual way, and may then be treated with the copper solution.

The cut sections are now well washed for twelve hours in water, and then placed in **hæmatoxylin solution**, made according to this formula: Hæmatoxylin 0·75 to 1 part; alcohol, 10 parts; distilled water, 90 parts; saturated solution of lithium carbonate, 1 part. The solution must not be quite fresh, and will not keep over one month. The sections of spinal cord, or medullary layers of the brain, require two hours immersion, and cortical substance twenty-four hours; the penetration of the stain is rendered more rapid if the solution is maintained at a temperature of 40° C. When

properly stained, the sections should exhibit a deep blue-black colour, and should be quite opaque.

The next step consists in washing in considerable quantities of water the stained sections, which should go on, it may be, for several days; at any rate, until the fresh washings exhibit no colour of the dye. The sections must now be placed in the following **differentiating solution**: Borax, 2 parts; ferrid cyanide of potassium, 2·5 parts; water, 200 parts, until there is a sharp definition of the gray and white matter in the tissue; this should take half an hour or more. The sections should now be thoroughly washed with water, **double-stained**, if desired, in eosin or borax carmine, dehydrated, cleared and mounted in the usual way.

(2) **Pal's Method.**—This is a modification of Weigert's in several particulars; it appears to possess distinct advantages over the earlier method.

(*a*) After hardening the material in chromic acid, bichromate of potassium, or some other preparation of chromium, sections should be cut and placed in Weigert's hæmatoxylin, preferably at a temperature of 38° C. for five or six hours, and until of a bluish-black colour.

(*b*) The sections should next be washed in water until no more colour comes out in the washings; a few drops of lithium carbonate solution may be added to the water used.

(*c*) The sections should next be placed in a 0·25 solution of potassium permanganate for fifteen to twenty seconds.

(*d*) Washed in water.

(*e*) Placed in **Pal's solution**, made thus: Oxalic acid, 1 part; potassium sulphite, 1 part; distilled water, 200 parts, for two to three minutes.

(*f*) Thoroughly washed in water.

(*g*) Double-stained in borax carmine, or picrocarmine.

(*h*) Dehydrated, cleared, and mounted.

(3) **Pal-Exner Method.**—The nervous material, cut into small segments, is placed fresh in a considerable quantity of 0·5 per cent. of osmic acid for two to three days, the osmic acid being changed daily, the material being cut after embedding in celloidin or paraffin. Sections are then treated with permanganate of potassium and Pal's solution, and according to the other process of Pal's method above described.

Structure.—The spinal cord consists of (1) *white matter*, (2) *gray matter*, and (3) *neuroglia*. Observe the structure of the spinal cord by means of a transverse section taken from the region of the cervical enlargement. In he first place examine the stained section

mounted in Canada balsam under a two-inch objective, or else with a hand magnifying-glass. Notice the roughly oval shape of the section.

The central and more deeply-stained portion is the gray matter. It is less granular than the white matter seen round the circumference. It offers for examination two crescentic masses with their concavities turned outwards, joined across the middle by a transverse portion (the gray commissure). In the centre is a small canal lined by a layer of columnar cells, which in young animals are ciliated. Each crescent presents (*a*) *the anterior horn*, or cornu, which is short and thick, and extends towards the anterior roots; (*b*) *the posterior horn* longer and more slender. In the concavity of each crescent the gray matter sends out processes which enclose portions of the white substance.

Surrounding the gray matter on all sides is the granular white matter. It is traversed by delicate bands of medullated fibres, which extend from the extremities of the gray matter towards the outside of the cord. These fibres are the anterior and posterior roots respectively of the spinal nerves. A delicate coat of fibrous tissue—the pia mater—encircles the cord, and sends into it numerous processes. The white substance is cleft vertically by a space—*the anterior median fissure*—which extends *nearly* down to the transverse portion of the gray matter. It divides that portion of the cord which is nearest to the bodies of the vertebræ. The white matter on the posterior aspect of the cord is divided by a somewhat similar space—*the posterior median fissure*. This fissure differs from the anterior in the fact that it is narrower, that it is deeper as it extends down to the gray commissure, and that it is usually occupied by delicate connective tissue. Laterally the surrounding pia mater penetrates the white matter at various points; these ingrowths are somewhat more marked in the neighbourhood of the anterior and posterior roots. This portion of the cord is therefore divided by antero-lateral and postero-lateral fissures into antero-median, antero-lateral, lateral, postero-lateral, and postero-median *columns*. At the bottom of the anterior fissure is a transverse band of white substance, called the anterior white commissure; it separates the fissure from the anterior gray commissure, which in turn is separated from the posterior gray commissure by the central canal. The posterior gray commissure lies immediately above the posterior median fissure.

Tracts of Degeneration.—By means of disease various tracts are mapped out in the cord, showing in many cases the course of the fibres. These tracts, as seen on transverse section with an ordinary magnifying glass, are as follows: In the postero-

lateral column, a little nearer the gray matter than the exterior, a somewhat oval or wedge-shaped area, consisting of fibres which have decussated in the anterior pyramid of the medulla and passed over to the lateral region. This area forms the *crossed pyramidal tract*, which diminishes in size from above downwards, and it can be traced as low as the third or fourth sacral nerve. Outside this a very narrow area—the *direct cerebellar tract*—can be traced down as far as the second lumbar nerve. On either side of the anterior fissure the uncrossed or *direct anterior pyramidal tract* diminishes downwards until it disappears about the middle of the dorsal region. On either side of the posterior fissure is the *posterior median column, or column of Goll*, traceable as low down as the middle of the dorsal region; outside this the *postero - external column, or column of Burdach*, can be distinguished. Between the anterior cornu and the exterior of the cord anterior to the direct cerebellar tract is *Gower's tract of* ascending degeneration. A small tract of descending degeneration may, under certain circumstances, be made out in the posterior external column, the ' comma tract,' and there is another ascending tract near the posterior cornu towards the outer side.

Under a somewhat higher power, such as a half-inch objective, it will be seen that the gray matter is composed of a finely granular matrix, enclosing numerous polygonal bodies which, in a well-prepared specimen, have many delicate branches. These bodies are the **ganglion cells.** They are largest and most numerous in the anterior horns. They are least numerous in the centre and in the commissures. The granular appearance of the white matter is due to the section of a large number of medullated nerve-fibres, which vary considerably in size, and are embedded in a matrix of neuroglia. In transverse section these fibres consist of circular masses with a more deeply-stained centre, representing the axis cylinder. In the anterior region the white matter is traversed by several bundles of non-medullated fibres, which form the anterior or motor root; whilst in the posterior region, the posterior or sensory root passes through it as a single band.

Under a still higher power, such as a $\frac{1}{6}$ inch objective, the various constituents of the cord may be considered in greater detail:

(1) The **white matter** consists of longitudinal medullated nerve-fibres, which in transverse section measure $\frac{1}{1500}$ to $\frac{1}{15000}$ of an inch in diameter, with connective tissue, and a special tissue called neuroglia. The fibres are finer in the posterior and postero-lateral columns than elsewhere. Each fibre can be seen to consist of an external coating derived from the neuroglia or supporting tissue of the nervous matter, which is composed of the minutely ramifying

cells, the branchings and anastomosing of which form the supporting meshwork of the nervous elements, which stains, and which surrounds an unstained portion, which again encloses a small central and deeply-staining portion. The unstained part is often arranged in concentric rings. The corpuscles of the neuroglia are seen here and there between the nerve-fibres.

(2) The **gray matter** is made up of small non-medullated fibres, which chiefly form a dense network, continuous with the roots of the nerves; part of these fibres are derived from the branches of the nerve-cells embedded in the network. It also contains fine medullated fibres (seen only, however, when Weigert's method has been used), naked axis cylinders, and neuroglia. The cells are of two kinds:

(i.) *Large*, *branched*, and *nucleated*, which are most numerous in the anterior cornua, especially at their upper and inner parts. They are also found at the inner part of the base (cervix cornu) of the posterior horn, forming the *posterior vesicular column*, which is best marked in the lumbar enlargement of the cord. Also in the concavity of the crescent is a group of cells, occupying a projection of gray matter there and called the *tractus intermedio-lateralis;* it is best marked in the dorsal region and elsewhere.

(ii.) Smaller cells scattered throughout the gray matter, but chiefly at the tip (caput cornu) of the posterior horn, in a finely granular basis, and among the posterior root fibres (*substantia gelatinosa cinerea* of Rolando).

Considerable stress is laid upon the supporting elements which exist in the spinal cord as well as in the remainder of the central nervous system. It appears to be of two kinds: (*a*) The connective tissue which forms the pia mater, which is made up of ordinary fibrous connective tissue, including many elastic fibres, and is very abundantly supplied with blood-vessels, of which it is, indeed, the special carrier. This material forms a complete investment for the cord, sending in processes of similar structure, which radiate inwards towards the gray matter, filling up the posterior fissure, dipping down, and lining the wider anterior fissure. From the trabeculæ sent in by the pia mater secondary processes pass off into the interior carrying blood-vessels; when they have become fine, these secondary processes join the second (*b*) or special supporting material of the tissue, to which attention has been already directed—viz., the neuroglia. This tissue was formerly looked upon as a variety of retiform tissue, but it appears that it differs considerably from all

such tissue. It is, indeed, made up entirely by the exquisitely fine branchings of cells laid down (cells of Deiters) in a homogeneous ground-substance, the cell-bodies being reduced to a very small size, and the nuclei being in many instances absent; but the neuroglia is found to yield not gelatin, as does most connective tissue, but a substance allied to keratin, and which is called **neuro-keratin**. Developmentally, too, the tissue differs from connective tissue proper, being derived from the epiblast and not from the mesoblast. The neuroglia forms a perivascular lymph-space for the blood-vessels, which are numerous but small.

The Peculiarities of Different Regions of the Spinal Cord.—The outline of the gray matter and the relative proportion of the white matter varies in different regions of the spinal cord, and it is, therefore, possible to tell approximately from what region any given transverse section of the spinal cord has been taken.

In the cervical enlargement the gray matter occupies a large proportion of the section, the gray commissure is short and thick, the anterior horn is blunt, whilst the posterior is somewhat tapering. The anterior and posterior roots run some distance through the white matter before they reach the periphery.

In the dorsal region the gray matter bears only a small relation to the white, and the posterior roots in particular run a long course through the white matter before they leave the cord; the gray commissure is thinner and narrower than in the cervical region.

In the lumbar enlargement the gray matter again bears a very large proportion to the whole size of the transverse section, but its posterior cornua are shorter and blunter than they are in the cervical region. The gray commissure is short and extremely narrow.

At the upper part of the conus medullaris, which is the portion of the cord immediately below the lumbar enlargement, the gray substance occupies nearly the whole of the transverse section, as it is only invested by a thin layer of white substance. This thin layer is wanting in the neighbourhood of the posterior nerve-roots. The gray commissure is extremely thick.

At the level of the fifth sacral vertebra the gray matter is again in excess, and the central canal is enlarged, appearing T-shaped in section; whilst in the *upper portion of the filum terminale* the gray matter is uniform in shape without any central canal.

THE BULB OR MEDULLA OBLONGATA.

Preparation.—The bulb is prepared for the microscope in a manner similar to that described for the spinal cord. Weigert's or

Pal's method of staining should be employed for some of the sections.

Structure.—Under a low power a transverse section through the bulb (i.), a little *below the apex of the calamus scriptorius*, shows the great depth of the anterior median fissure, with the comparative shallowness of the posterior fissure. The latter fissure is bounded on either side by the *fasciculus gracilis*, which is the continuation upwards of the posterior median columns of the spinal cord. The upward prolongation of the postero-lateral column, which has here expanded into the wedge-shaped *fasciculus cuneatus*, is situated more anteriorly than the fasciculus gracilis; and still more in front is a new band of fibres known as the *tubercle of Rolando*. The *olivary body*, which is dentate in section, is yet more anterior, and is separated from the *pyramid* by a bundle of fibres, which represents *the root of the hypoglossal nerve*.

The higher power will show that the anterior and lateral regions of the gray matter are here arranged in a *reticular formation*, caused by the intersection of nerve-fibres running in longitudinal and transverse directions. Numerous multipolar ganglion-cells are scattered throughout the reticular formation.

(ii.) A section through the bulb *at the level of the fourth ventricle* shows that the anterior median fissure still remains as a deep cleft, whilst the posterior median fissure has expanded into the space which represents the fourth ventricle. A layer of gray matter, containing numerous ganglion-cells, forms the floor of the ventricle. The group of ganglion-cells nearest to the middle line upon either side is the nucleus of the *fasciculus teres*. A little external and anterior to it—*i.e.*, deeper in the gray matter—is a collection of large ganglion-cells, forming the *nucleus of the hypoglossal nerve*, from which the nerve-roots may be seen passing through the reticular formation to the point where they leave the bulb, between *the pyramid* and *the olivary nucleus*. Still more externally are other groups of ganglion-cells, from which *the vagus* arises. This group of cells is partially subdivided by a bundle of fibres known as *the fasciculus solitarius*, which appears circular in transverse section; of the two groups, the more central and deeper is the larger. The root of the nerve passes outwards through *the reticular formation*. The *nucleus gracilis* and the *nucleus cuneatus* lie still more externally; they are portions of gray matter which extend respectively into the fasciculus gracilis and fasciculus cuneatus. The *ascending root of the fifth nerve* is situated laterally as a well-defined bundle of white fibres.

THE CEREBELLUM.

Preparation.—The cerebellum should be hardened in a 2 per cent. bichromate of potassium solution.

To stain specimens of it, the following method is recommended. Thin sections should be left in a 0·01 per cent. solution of eosin for twelve hours, and then, after washing in distilled and slightly acidulated water, should be placed in a weak solution of aniline green for fifteen or twenty minutes, being afterwards passed rapidly through the ordinary reagents, and mounted in Canada balsam.

Structure.—It is divisible into (*a*) Cortical gray; and (*b*) Internal white substance.

(*a*) **The Cortex** is composed of:

(1) *The molecular layer*, the most external, consisting of a nerve network containing small multipolar ganglion-cells. The fibres of the network in the more superficial portions are nearly vertical to the surface; they are derived partly from the neuroglia, partly from the processes of the cells of Purkinje.

(2) A single layer of large, spindle-shaped ganglion-cells $\frac{1}{800}$ to $\frac{1}{1000}$ inch in diameter, *Purkinje's cells*. Each cell possesses one branched process, which extends into the molecular layer, where it branches dichotomously, some of the finest ramifications looping backwards to terminate in the granular layer, and an unbranched axis-cylinder process passing downwards. The cells lie in a pericellular space, and each consists of a minute network of fibrils extending into the branched processes. The nucleus is spherical and oval.

(3) *The granular layer*, containing a network of minute fibrils and dense groups of granule-like corpuscles. These corpuscles average $\frac{1}{4000}$ to $\frac{1}{2500}$ inch in diameter.

(*b*) *The* **medullary centre**, or *internal white substance*, consists of nerve-fibres arranged in parallel or interlacing bundles.

The **neuroglia** of the white matter contains rows of small cells, each with a spherical nucleus, between bundles of nerve-fibres.

The **blood-vessels** of the gray matter pass from the pia mater in a vertical or oblique direction, and anastomose into a uniform network. The blood-vessels of the white matter form a network with longitudinal meshes. The vessels lie in lymph channels, *the perivascular lymphatics* of His.

THE CEREBRUM.

Preparation.—Having carefully removed the pia mater, place small pieces from different parts of the human cerebrum in a 2 per cent. solution of ammonium bichromate for two days, after which transfer to weak, and finally to strong spirit. Take care to get vertical sections. Stain some in aniline blue-black, and others by Weigert's or Pal's method.

Structure.—Get a general view of the structure with a $\frac{1}{2}$ or $\frac{3}{4}$ inch lens, and then use a $\frac{1}{5}$ objective. With the former it will be seen that the cerebral convolutions are divisible into (*a*) Cortical gray portion, and (*b*) Medullary white substance.

With the high power the (*a*) **Cortical gray portion** is seen to be composed of :

(1) *An external layer*, containing a few small cells with fine processes embedded in a considerable quantity of neuroglia. This layer composes about $\frac{1}{10}$ of the whole thickness of the gray substance.

(2) *A second layer* of small, densely-aggregated, pyramidal cells, provided with branching processes. This layer is of nearly the same extent as the previous one.

(3) *A third layer* of greater width, somewhat paler than the first and second layers ; it is composed of large and small pyramidal cells, arranged with their apices turned towards the surface of the convolution. The larger cells average $\frac{1}{1800}$ inch across their base. The cells are arranged in groups, and are separated from each other by bundles of radiating nerve-fibres, each bundle being about $\frac{1}{1500}$ inch in diameter. The pyramidal cells send downwards three processes, of which the middle one forms an axis cylinder. Both cells and processes are striated longitudinally, and generally contain a little yellowish pigment.

(4) *A fourth layer* is somewhat narrower than the preceding; it consists of small, irregularly-placed, granule-like corpuscles, with delicate processes. The cells are less distinctly separated into groups.

(5) *The lowest layer* is of considerable width ; it contains, in addition to cells resembling those of the fourth layer, fusiform cells arranged vertically at the summit of a gyrus, but parallel to the surface of a sulcus. This layer gradually blends with

(*b*) **The White Substance**, which is composed essentially of white nerve-fibres, smaller than those of the spinal cord, and

with an average diameter of $\frac{1}{10000}$ inch. In the neighbourhood of the cortex, a few non-medullated fibres can be seen.

(*c*) **The Neuroglia** is formed of a homogeneous matrix, in which lie numerous fibrils, connected into a network. With this network the branched nucleated cells of Deiters are connected, as in the spinal cord.

THE SYMPATHETIC SYSTEM.

Preparation.—Harden (1) portions of the Gasserian ganglion from the sheep in chromic acid and spirit.

(2) Pieces of the sympathetic nerve from the neck of an ox, in the manner recommended for medullated nerve-fibres.

(3) Pacinian corpuscles by snipping out pieces of the mesentery or meso-rectum of the cat, in which they may be seen as small bodies embedded in the fat; isolate them with needles, treat with osmic acid, stain in picrocarmine and mount in glycerine.

(4) Meissner's and Auerbach's plexuses, situated the one in the submucous coat, and the other between the muscular coats of the intestine, are best prepared from the rabbit. A piece of intestine, 3 inches in length, is distended with the juice of a fresh lemon, the ends being ligatured; it is allowed to remain in the lemon-juice for about five minutes. The ligatures are then removed, and it is washed thoroughly in water, and filled with a 2 per cent. solution of gold chloride; it is again ligatured, and is then suspended for half an hour in a 1 per cent. solution of gold chloride, washed thoroughly, and transferred to a 24 per cent. solution of formic acid to reduce the gold, the preparation being kept in the dark. After reduction of the gold, the intestine is of a rich reddish-brown colour; it should be again washed. Peel off strips of the outer muscular coat with forceps, and mount in glycerine. Auerbach's plexus will be seen. The plexus of Meissner is demonstrated by treating the intestine as before, and afterwards inverting it and removing the mucous coat, when portions of the submucous coat may be picked off from the muscular coats, and mounted in glycerine.

Nerve-endings are best seen in the rabbit's œsophagus. Excise the œsophagus of a small rabbit, place a ligature round its lower end, and distend it to a moderate extent with a normal saline solution; ligature the upper end. Place the œsophagus thus prepared in a large test-tube filled with a $\frac{1}{5}$ per cent. solution of osmic acid, and leave it there for three or four minutes. At the end of this time take out the œsophagus, lay it open longitudinally, and put it for two or three hours in an excess of normal saline solution.

Tear off pieces of the muscular coat, mount them in glycerine, and examine. The course of the nerves is readily made out, and the outlines of the nerve-endings is clearly visible.

Nerve-endings may also be demonstrated by staining with methylen-blue. This method consists of two distinct parts, the first being the treatment with the blue pigment, the second that of its fixation with picrate of ammonia. The solution of methylen-blue is made by dissolving 1 grm. of the pigment in 300-400 c.cm. of a $\frac{1}{2}$ per cent. solution of salt. The picro-glycerine is prepared from a cold saturated solution of picrate of ammonia by diluting with an equal quantity of pure glycerine. The animals may be injected through a blood-vessel with the methylen-blue solution; but it is usually more convenient to put small pieces of perfectly fresh tissue for ten minutes in the methylen-blue. They should then be washed in a $\frac{1}{2}$ per cent. salt-solution, and afterwards passed through the picro-glycerine, when they are at once ready for examination.

General Characters. — **The ganglion-cells** are of various shapes and sizes, generally smaller than the cells of the cerebro-spinal ganglia; they possess a capsule, and consist of a network of fibres. There is usually one large, excentric, oval nucleus, which may, however, be double. The cells possess one or more processes, which are continuations from the cell-substance, and are invested by a prolongation from the hyaline sheath. According to the number of processes, the cells are unipolar, bipolar, or multipolar. In the frog the bipolar cells are peculiar, since one of the processes appears as a 'spiral fibre' twisted round the other process, or 'straight fibre.'

The nerve-trunks contain medullated fibres similar to those already described, and non-medullated, or fibres of Remak. Remak's fibres are pale, finely fibrillated axis cylinders, invested with a hyaline sheath of Schwann provided with nerve-corpuscles.

The Pacinian bodies are oblong corpuscles connected with a medullated nerve-fibre which represents its stalk. The corpuscle consists of a number of concentric capsules. Each capsule is composed of a hyaline basement membrane, which is probably elastic, and in which are embedded fine connective-tissue fibres. On the inner surface of the basement-membrane is a layer of flattened nucleated endothelial cells, which are visible after treatment with nitrate of silver. In the centre of the corpuscle is a clear mass in which lies the axis cylinder of the perforating nerve-fibre. The axis cylinder generally breaks up into two or three branches, or it may terminate in a bud or in a pointed or fringed

extremity. The corpuscles contain, between the capsules, capillary blood-vessels and a few plasma-cells.

The nerve plexuses consist of a meshwork of flat nerves, each of which is ensheathed in a delicate endothelial membrane. At the nodal points are groups of ganglion-cells, which vary in size and shape; the larger ones possess a capsule and processes. The plexus of Meissner consists of a larger and less regular meshwork than that of Auerbach. The two plexuses are connected by branches which pass through the circular layer of muscle. Each plexus gives off branches which supply the surrounding tissues.

CHAPTER VIII.

BLOOD-VESSELS.

Of three kinds: (*a*) Arteries; (*b*) Veins; and (*c*) Capillaries.

(*a*) **Arteries.**—*Longitudinal* and *transverse* sections of a medium-sized artery and vein which have been hardened in a 1 per cent. solution of potassium bichromate should be stained in hæmatoxylin, prepared, and mounted in Canada balsam.

Good transverse sections of small arteries and veins may be seen in sections of various organs—*e.g.*, the tongue, liver, or heart.

In order to show their endothelial coat, the fresh vessels should be distended with a ¼ per cent. solution of silver nitrate for ten minutes. After washing in distilled water, thin sections should be shaved off the internal surface with a razor, and should be mounted and examined in glycerine.

The structure of the arteries is as follows: they consist of (except those of minute size) three coats:

(1) Internal coat, consisting of: (*a*) *An endothelial layer*, forming the lining of the vessel, of thin elliptical or irregularly polygonal cells, often lanceolate, with nuclei and nucleoli; (*b*) *a sub-endothelial layer* of delicate connective tissue, with branched corpuscles; (*c*) *elastic layers* of longitudinal elastic networks and 'fenestrated' membrane.

(2) Middle coat chiefly consists of circular *unstriped muscle fibres*, mixed with *elastic fibres*, and a sparse amount of connective tissue.

(3) External coat (*tunica adventitia*) chiefly consists of fine and closely-felted bundles of *connective tissue*, with longitudinal elastic tissue between them.

In the largest arteries, the middle coat consists of alternate layers of elastic tissue and unstriped muscle. In the smallest arteries (arterioles) the coats are reduced to a muscular, principally of circular fibres, and a lining endothelium.

(*b*) **Veins.**—The structure of the veins is similar to that of the arteries, with these differences: The elastic tissue of the internal coat seldom occurs in the form of fenestrated membranes. The middle coat is thinner, contains less muscular tissue and more white connective tissue. The external coat of some veins has a considerable amount of unstriped muscular fibre. The valves, when they are present, consist of folds of the internal coat covered on each surface with endothelium. Each fold contains a few muscular fibres and a little connective tissue derived from the middle coat.

(*c*) **Capillaries.**—Good specimens of capillary vessels may be obtained from the pia mater. For this purpose the brain of some animal, such as a cat or dog, should be left for two days in a 2 per cent. solution of potassium bichromate; then the pia mater may be stripped off in pieces, stained, and mounted in the usual manner. Capillaries are well seen also in the mesentery of a cat or other animal.

In structure, the walls of the capillaries proper may be seen to be formed entirely of a simple epithelial layer of flattened lanceolate cells, joined edge to edge, and continuous with the layer which lines the arteries and veins. The larger capillaries have an outside structureless or finely-fibrillated coat. In rather larger vessels (small arteries and veins) there is added, outside the epithelium, a thin layer of unstriped muscular fibre.

CHAPTER IX.

THE ALIMENTARY CANAL AND ITS APPENDAGES.

THE TONGUE.

Sections of the tongue of a rabbit or cat which has been hardened in equal parts of ½ per cent. chromic acid and spirit, or in a saturated solution of picric acid, should be stained with hæmatoxylin, and prepared in the usual manner.

Sections of the *papilla foliata* from the side of the base of the rabbit's tongue should also be treated in a similar manner. The tongue furnishes excellent material for double or triple staining.

Structure.—The tongue, for histological purposes, may be considered to consist of a mucous membrane covering striped muscles and glands.

The mucous membrane of the dorsal surface is elevated into numerous papillæ, which are of varying shape. Each papilla is covered by stratified epithelium, the superficial layers of which in the carnivora are converted into dense plates of chitinous material. The papillæ, according to their shape, are known as the filiform, the fungiform, and the circumvallate.

(*a*) **The filiform papillæ** are the most numerous, and are found upon the anterior and lateral surfaces of the organ. They are minute elevations of the corium covered by epithelium, their apices being frequently subdivided into secondary papillæ.

(*b*) **The fungiform papillæ** resemble in shape unexpanded mushrooms or 'puff-balls.' They consist, like the filiform papillæ, of areolar tissue enclosing a loop of capillaries, and they are covered with squamous epithelium. These papillæ are most numerous at the apex and near the margins of the tongue.

(*c*) **The circumvallate papillæ** are situated at the posterior portion of the dorsal aspect of the tongue, and are much larger than either the filiform or fungiform

papillæ. In section they appear as flat-topped elevations of the mucous membrane, with a deep depression upon either side. The duct of a serous gland frequently opens into the bottom of the depression. They are covered with flattened epithelium like the other varieties of papillæ, but at their sides some of the epithelial cells have become modified into *taste-buds*.

(*d*) **The papillæ foliatæ** are elongated oval patches seen upon either side of the base of a rabbit's tongue. They consist of a number of parallel invaginations of the mucous membrane. The epithelium thus invaginated contains numerous ovoidal or flask-shaped bodies identical with those found in the human circumvallate papillæ: they are *the taste-buds*. Each of these bodies consists of a layer of flattened epithelial cells, arranged round, and forming a covering for, a bundle of central cells. The central cells are spindle-shaped, one extremity projecting through the aperture left by the external cells, whilst the other extremity is branched and extends downwards, and probably becomes connected with a terminal fibre of the glosso-pharyngeal nerve.

Numerous *mucous secreting glands* are found in the tongue. Of these the largest are the glands of Nuhn, situated at the tip. *Serous glands* are also present; they open into the trenches of the circumvallate papillæ. *Lymphoid tissue* is found in large quantity in the mucosa at the posterior part of the tongue, in some cases forming distinct follicles; numerous crypts and recesses, too, are found, the walls being studded with nodules of lymphoid tissue.

The mucous membrane on the under surface of the tongue consists of thinner layers of stratified pavement epithelium, with a somewhat dense mucosa. On the dorsum of the tongue the submucous coat is incomplete and scanty, though it contains a large number of blood-vessels.

The muscular portion of the tongue consists of longitudinal, transverse, and vertical fibres of striated muscles; considerable quantities of fat and gland-tissue are found between the bundles of muscle.

THE TEETH.

The teeth should be examined (*a*) By means of sections of the hard tooth. These sections are thus made: The tooth is ground down on both sides till it is quite thin, it is then mounted in hard Canada balsam, so that air is retained in the various cavities.

(*b*) By means of sections of the softened tooth: The tooth is placed in a 10 per cent. solution of hydrochloric acid till it is quite soft, and is then immersed in spirit; by these means the structure of the dentinal substances may be investigated; or the tooth (preferably broken across) may be placed in a saturated solution of picric acid until it is quite soft; the hardening being completed in spirit, the spirit being changed as long as it becomes tinged with the picric acid. This method of preparation will preserve the pulp and odontoblasts.

To demonstrate the pulp, a freshly-extracted tooth should be broken and immersed in osmic acid $\frac{1}{5}$ per cent. for twenty-four hours.

To show the development of teeth, the lower jaw of a fœtal rat, dog, or kitten, from which the muscles have been removed, should be placed in $\frac{1}{6}$ per cent. chromic acid for seven days, then removed to weak spirit for twenty-four hours, and finally to strong spirit till required.

(*c*) The dentinal sheath lining the tubules may be isolated by boiling for ten minutes in strong sulphuric acid.

In each case hæmatoxylin staining should be used.

Structure.—(1) A tooth consists most externally, and above the gum, of **enamel.** In the recently cut tooth, which has not been much used, there exists above the enamel a covering of epithelial or horny nature (Nasmyth's membrane), which is structureless, and has a thickness of $\frac{1}{30000}$ to $\frac{1}{10000}$ inch. The enamel covers the crown and neck of the tooth; it is an epithelial product, consisting of closely aggregated polyhedral cylinders forming the enamel fibres (prisms or columns). These fibres are crossed by a number of darker lines, arranged in concentric layers, 'contour lines.' In transverse section the enamel fibres are seen to be six-sided prisms, with an average diameter of $\frac{1}{5000}$ inch.

(2) **The Dentine**, or ivory, covers the body and root of the tooth; in the crown it lies immediately below the enamel. The dentine consists of a compact bone-like substance, which contains no bone corpuscles, and is permeated by dichotomously dividing canals, the dentinal tubules, which average $\frac{1}{4500}$ inch in diameter. The dentinal tubules run perpendicularly to the surface of the pulp cavity, into which they open by their lower extremities. The tubules present a proper wall, consisting of a membranous tube, and each contains a process of protoplasm from the superficial layer of the pulp-cells. Examined under a lower power, the tubules are seen to form two or three gentle curvatures, which give rise, when a number of tubules are seen together, to a series of concen-

tric lines (*lines of Schreger*). Certain interglobular spaces, due to imperfect deposition of salts, are also frequently seen in the dentine.

(3) **The crusta petrosa**, or cement, invests the portions of the tooth which are not protected by enamel. It closely resembles bone in its histological appearances, except that the lacunæ and canaliculi are larger and more irregular. When the cement is very thick, it may contain vascular channels, which are comparable with Haversian canals. The perforating fibres of Sharpey are present in considerable numbers in this layer.

(4) **The pulp** occupies the central cavity of the tooth; it consists of jelly-like connective-tissue, in which run nerves and blood-vessels. The outermost layer of cells, forming the pulp, are elongated in form, the bodies somewhat resembling columnar epithelium cells. This layer forms the membrana eboris, of which each cell is an odontoblast. The odontoblasts send off one or more processes, which run in the dentinal tubules; processes which connect the cells together laterally, and processes which unite the cells to others, lying more deeply.

(5) **Osteodentine**, or secondary dentine, is the hard substance deposited on the inner surface of the dentine, which is produced by the gradual conversion of the pulp.

Development.—(1) The first rudiment of a tooth appears as a solid prolongation of the stratified epithelium, which grows downwards from the surface into the mucous membrane. This process of epithelium is the *primary enamel organ.*

(2) The enamel organ becomes invaginated at its deep end by a mass of tissue derived from the mucous membrane, called the embryonal tooth papilla. The primary enamel organ is thus converted into the **enamel cap** covering the tooth papilla.

The **papilla** is vascular, and is composed of a network of nucleated cells; it forms the pulp, and by means of its odontoblasts produces the dentine.

The **odontoblasts** appear on the papilla as a peripheral stratum of large cells arranged vertically.

The **dentine** is formed by the calcification of the substance which surrounds, and is perhaps derived from, the distal extremities of the odontoblasts, whilst

The **dentinal fibres** are processes of the odontoblasts.

The **tooth sac**, or the mucous membrane which immediately surrounds the enamel cap and tooth papilla, gradually grows over the former, and separates it from its connection with the surface epithelium.

The **enamel cap** consists externally of (*a*) columnar cells, more internally of (*b*) polyhedral cells, followed by (*c*) flattened epithelial cells in the centre, and again of (*d*) polyhedral, with (*e*) columnar cells most internally—*i.e.*, nearest to the tooth papilla.

The enamel cap is limited both externally and internally by a membrana propria. The enamel cap becomes divided into an inner and outer membrane by the transformation of the middle layer into a transparent tissue. *The inner membrane* is composed of columnar cells, the enamel cells, in contact with the dentine; each is a long hexagonal prism, and is nucleated at its lower part. Outside the layers of enamel cells are one or more rows of small polyhedral cells, forming the stratum intermedium. *The outer membrane* is composed of several layers of epithelial cells.

The **enamel** is formed by the enamel cells of the inner membrane elongating at their distal extremities; the elongated portion is transformed directly into enamel.

The cells of the stratum intermedium are used for the regeneration of the enamel.

The cells of the outer epithelium produce the enamel cuticle.

The **cement** is formed from the tissue of the tooth sac in exactly the same way as sub-periosteal bone is developed.

During the stage of the primary enamel organ (*a*) a lateral process grows out from the epithelial cells, which represents the rudiment of the enamel organ of the permanent tooth (*sac of reserve*).

The permanent teeth are developed on exactly the same plan as the deciduous set.

SALIVARY GLANDS.

Sections of the submaxillary gland of a cat or dog, of the parotid of a rabbit or dog, and, if obtainable, of the human parotid, sublingual, and submaxillary glands, should be made in various directions after hardening in absolute alcohol. The sections should be stained with Ehrlich's hæmatoxylin. Pieces of salivary glands, both actively secreting and also resting, should be treated with osmic acid saturated in paraffin wax, cut, and mounted in ribbons.

Structure.—The salivary glands are compound tubular glands enveloped in an incomplete capsule.

The capsule consists of fibrous tissue, which sends septa into the substance of the gland. The septa support the blood-vessels, lymphatics, nerves, and ganglia, and they divide the glandular substance into lobes and lobules.

Each **lobule** is made up of the convolutions of the main division of a duct bound together with connective-tissue. The convoluted

parts are lined by and almost filled with a single layer of columnar cells (*salivary cells*) enclosing a nucleus. These cells, when isolated, are not unfrequently found to be branched. The basement membrane of the tubes consist of branched and flattened cells, and between it and the salivary cells are found, here and there, in the mucous variety of salivary glands (see below), granular semilunar bodies (the **Crescents of Giannuzzi**). The smallest divisions of the ducts have a relatively small lumen, and are lined near the convolutions with flattened epithelium, and then with nucleated columnar cells, which present a longitudinal striation. The larger ducts acquire an external coating of connective-tissue, and are lined with a single layer of columnar epithelium. In the walls of the largest ducts are unstriped muscular fibres.

The salivary glands are divided into (*a*) **Mucous glands**, in which the alveoli are large, and the contents are:

(1) **Mucous cells**, transparent and columnar, with their pointed extremities applied to the membrana propria; the cells are imbricated; the nucleus is much compressed, and is near the membrana propria.

(2) The **Crescents of Giannuzzi**, semilunar groups of cells situated here and there between the mucous cells and the membrana propria. The cells are small and polyhedral, with a spherical nucleus. The submaxillary and orbital glands of the dog and the sublingual gland of man are of the mucous type.

(*b*) **Serous glands**, in which the lumen of each alveolus is small, and the epithelium consists of a single layer of short columnar cells, each with a spherical nucleus situated at the periphery of the cell. The parotid gland and the greater part of the submaxillary gland of man and the guinea-pig, as well as the submaxillary and orbital glands of the rabbit, are serous glands.

(*c*) **Muco-salivary glands**, such as the submaxillary gland of man and the guinea-pig, are formed by the mixture of the mucous and serous types of glands.

In the connective-tissue binding the lobules together many nerve-cells may be seen.

THE TONSILS.

Sections made from a tonsil which has remained for a week in $\frac{1}{6}$ per cent. solution of chromic acid, and subsequently in spirit, should be stained with hæmatoxylin and mounted in Canada balsam. An enlarged tonsil, which has been removed from a child, will answer the purpose if no other material is attainable.

Structure.—A tonsil consists of an elevation of the mucous membrane, presenting upon its surface fifteen orifices leading into crypts or recesses, in the walls of which are placed nodules of lymphoid tissue. These nodules are enveloped in a less dense lymphoid or adenoid tissue, which reaches to the mucous surface. The mucous surface, including the crypts, is usually covered with stratified squamous epithelium, and may present rudimentary papillæ, which are then formed of adenoid tisssue. The tonsil is bounded by a fibrous capsule. Into the crypts open a number of ducts of mucous glands.

THE ŒSOPHAGUS.

Small pieces of the upper and lower parts of the œsophagus of a dog should be hardened in chromic acid and spirit, and sections, both transverse and longitudinal, should be prepared and mounted.

Structure.—The œsophagus consists of three coats:

(1) An **external** or **muscular coat**, arranged in two layers, longitudinal and circular, the former or external layer at the commencement being disposed in three fasciculi, one in front and one on each side. At the upper end of the œsophagus the muscular coat is red, and consists of striated muscle; lower down, in most cases, it becomes paler, and the fibres are chiefly unstriated.

(2) A **submucous coat**, which is made up of areolar tissue, and contains mucous glands (which are best seen in sections from the lower part) and whose ducts pass inwards to open on the mucous membrane.

(3) A **mucous coat**, which is firm and wrinkled, provided with minute papillæ, and covered with a thick layer of stratified squamous epithelium. It is separated from the submucous coat by a layer of unstriated muscular fibres, longitudinally arranged, which is partially imperfect as a layer above, but complete below (*muscularis mucosæ*).

The **arteries** situated in the submucous tissue give off branches, which form a network of capillaries in the upper part of the mucous coat; from this network loops are given off to supply the papillæ.

THE STOMACH.

The stomach of a cat or dog should be used. After removal from the recently killed animal, the organ should be turned inside out, and washed with a gentle stream of weak bichromate of potash or

spirit. Pieces of the mucous membrane from different parts should be snipped off with a sharp pair of scissors, and placed in chromic acid and spirit, picric acid, or in strong alcohol. Pieces of the whole thickness of the viscus should also be cut off; these may be hardened in weak chromic acid, or in the chromic acid and spirit mixture.

Sections must be cut both vertical and parallel to the surface at different depths.

To demonstrate the **structure of the glands** of the mucous membrane, some sections from each part should be stained in logwood, and others in a 0·5 per cent. solution of aniline blue, as the aniline will stain the parietal cells very deeply, and so differentiate them from the cubical central cells. This is especially evident in horizontal sections of the peptic glands. The aniline tinted specimens may be passed through slightly acidulated water, as usual in aniline staining, before they are placed in spirit.

Structure.—The stomach is made up of four coats:

(1) The **mucous** or **internal coat** is smooth, soft, pulpy, and pink in colour, becoming gray soon after death. It is thickest at the pylorus, thinnest at the great curve, and is loosely connected with the muscular coat by means of the submucous tissue, so presenting temporary ridges (rugæ) when the organ is contracted. It consists almost entirely of small tubular glands, arranged close to and parallel with each other, varying in diameter from $\frac{1}{500}$ to $\frac{1}{360}$ of an inch, and in length from $\frac{1}{60}$ to $\frac{1}{20}$ of an inch, lined to a variable extent by columnar epithelium, which also covers the whole of the mucous membrane.

The tubular glands are for the most part simple, except near the pylorus, where they become larger, longer, and branched. The glands consist of a basement membrane formed of branched stellate cells joined edge to edge, which send processes on the one hand to join the retiform tissue of the mucous membrane, and on the other to support the gland-cells. The glands are of two kinds, differing chiefly in the character of the cells and of their secretion.

The one, the so-called '**mucous** or **pyloric** glands,' are often branched, are confined to the pylorus, and are lined throughout by columnar epithelium; but towards the 'fundus,' or closed extremity of the gland, the cells tend to become cubical.

The other, the **peptic** glands, are distributed through-

out the whole of the mucous membrane, except at the pylorus, but are most typical perhaps towards the cardiac end; they are less often branched, but two glands generally open into one duct, which occupies a third of the whole length of the gland. The lower end, or fundus, is somewhat dilated, and sometimes slightly curved. The duct is lined with columnar epithelium; its middle third contains **two distinct kinds of cells**; outside, large granular cells with small nuclei, bulging out the basement membrane, and making irregular the outline of the tubes, called the **parietal cells**; and inside, a layer of smaller finely granular cubical cells, **central cells**, which bound a small lumen. In the lower third, or fundus, the parietal cells do not form a continuous layer, but occur here and there irregularly. In this locality the remainder of the tube is filled with cubical central cells, which leave a very small lumen unoccupied. The cubical cells closely resemble those lining the fundus of the pyloric glands.

Between and beneath the glands is a quantity of delicate connective-tissue, forming the mucous membrane proper, which here and there is collected into small masses somewhat resembling the solitary follicles of the intestine. A double layer (circular and longitudinal) of unstriated muscles (*muscularis mucosæ*) separates the mucous membrane from the submucous coat.

(2) The **submucous coat** consists of areolar tissue with some fat, together with blood-vessels and lymphatics; small nerve ganglia and fibres are also found in it.

(3) The **muscular coat** consists of three layers of unstriated fibres, externally of longitudinal, then of circular, and internally of oblique fibres; the circular layer is the only complete one. Between the layers may be found *plexuses of nerves*.

(4) The **serous coat** is the peritoneal covering of the organ.

The **arteries**, after penetrating the muscularis mucosæ, break up into capillaries in the mucous membrane, which form a more or less elongated meshwork around the glands. Near the surface the meshwork is very dense, and forms a well-marked superficial layer beneath the epithelium.

THE SMALL INTESTINE.

To study the **epithelium in the fresh state**, a scraping from the mucous membrane of the intestine of a recently killed animal may

be teased and mounted in saline solution, after 24 hours' immersion in a 2 per cent. solution of potassium bichromate.

For the purpose of studying the **relations of the various structures** in the mucous membrane—*e.g.*, villi, Brunner's and Lieberkühn's glands, Peyer's patches, etc.—small pieces from each part of the intestine of the cat, dog, or rabbit, should be hardened in weak chromic acid or chromic acid and spirit mixture, and cut into sections in various directions.

To demonstrate the **large lymphatic sinus** surrounding the follicles comprising a Peyer's patch, the ileum is used, and the sinus is injected with Berlin blue by the puncture method (p. 61), whilst a 0·5 per cent. solution of silver nitrate will demonstrate the endothelial lining of the vessel.

To demonstrate **the absorption of fat** by the villi, a part of the intestine of an animal recently fed on fatty food should be ligatured and placed in Müller's fluid; in about a week's time pieces may be placed in osmic acid for twenty-four hours, and should afterwards be replaced in the solution.

For the preparation of **Meissner's plexus and the ganglia of Auerbach,** see p. 120.

Structure.—The small intestine consists of four coats:

(1) The **mucous coat** possesses:

(*a*) **Valvulæ conniventes**, which are large, naked-eye permanent folds or crescentic projections running transversely to the axis of the intestine, and containing the submucous coat. They first appear in the duodenum, not far from the pylorus; are largest in the duodenum and upper half of the jejunum, and then gradually become smaller, until they disappear about the middle of the ileum.

(*b*) **Villi** are small processes, closely set on every part of the small intestine, over the valvulæ conniventes, as well as between them. They are conical and flattened in form, sometimes cylindrical, or with the free end clubbed. They are largest in the duodenum and jejunum, in length varying from one-fourth to one-third of a line; but smaller, shorter, and fewer in the ileum. They consist of projections of the mucous membrane, covered with columnar epithelium, and enclosing blood-vessels, lymphatics, and the muscularis mucosæ, bound together by fine retiform tissue, which also forms the basement membrane and encloses numerous lymphoid corpuscles.

(*c*) **Crypts of Lieberkühn** are very numerous small tubular glands, existing everywhere in the small intestine; they are lined with columnar epithelium.

(*d*) **Brunner's glands** are smaller compound tubular glands found in the first half of the duodenum; lying in their submucous coat, their ducts pass through the mucous coat.

(*e*) **Peyer's Patches** are follicular lymphatic glands which occur agminated into oblong patches in the ileum, especially at its lower part, lying in the long axis of the intestine opposite the attachment of the mesentery.

(*f*) **Separate lymphoid follicles** also are found everywhere in the small intestine, both between and upon the valvulæ conniventes.

(2) The **submucous coat** resembles that of the stomach, as does also

(3) The **muscular coat**; but this in the intestine has no oblique fibres.

(4) The **serous coat** of the duodenum is partially incomplete.

The **arteries** passing through the muscularis mucosæ give off numerous capillaries, which form a network around the crypts of Lieberkühn; the artery which passes into the villus generally ascends to the apex, and then breaks up into a dense plexus of capillaries, which spreads over the apex and base. The capillaries are always situated in the periphery next the epithelium. There are generally one or two veins developed from the capillaries of the villus. *The* **lymphatic** of each villus consists of a single central vessel, or of two such vessels anastomosing with each other.

THE LARGE INTESTINE.

The large intestine is prepared for examination in the same way as the small intestine, to which it is similar in structure, but with the following differences:

(1) The **mucous coat** has neither villi, Brunner's glands, nor valvulæ conniventes, and its crypts of Lieberkühn are longer, more numerous, and are placed more closely together. The lymphoid follicles are always solitary.

(2) The **muscular coat**. In the colon and cæcum, the longitudinal layer is collected into three flat bands, which causes the intestinal wall to be puckered into 'sacculi.'

(3) The **serous coat** of the colon and upper part of rectum is developed into small projections containing fat (appendices epiploicæ). It is incomplete in some parts.

THE PANCREAS.

The pancreas of a recently-fed dog should be taken, cut into pieces about half the size of a small hazel-nut, and placed at once in absolute alcohol to harden. For the sake of comparison, another pancreas from a fasting dog should be treated in a similar manner.

Some small pieces should also be treated with osmic acid. Sections should be stained and prepared in the usual way. Unless hardened in alcohol or osmic acid, the gland is very likely to become useless (from self-digestion ?) for microscopic purposes.

Structure.—The capsule and septa, as well as the blood-vessels, nerves, and lymphatics, are arranged as in the salivary glands; the gland proper is, however, looser and softer, and the lobes and lobules are less compactly arranged.

The **larger ducts** possess a very distinct lumen, and a membrana propria lined with columnar epithelium cells which are longitudinally striated, but are shorter than those found in the ducts of the salivary glands.

In the **smaller ducts** the epithelium is short and the lumen is smaller.

The **intermediary ducts** opening into the alveoli possess a distinct lumen, with a membrana propria lined with a single layer of flattened elongated cells.

The **alveoli** are branched and convoluted tubes, with a membrana propria and a single layer of columnar cells. The cells consist of an outer part nearest the membrana propria, which is homogeneous, and stains the more deeply; and an inner, more granular, and less readily stained portion. The alveoli do not contain the semilunes seen in mucous salivary glands, and have no distinct lumina, their places being occupied by fusiform or branched cells. Collections of smaller and less deeply staining cells are seen in greater or less number throughout the gland.

THE LIVER.

Small portions of the fresh liver of a pig, rabbit, or puppy, should be steeped for four or five days in a 2 per cent. solution of potassium bichromate, and then for one or two days in methylated spirit. Sections should afterwards be cut and treated as usual.

It is well, also, to mount sections of a liver which has been injected through the portal vein with 2 per cent. solution of Berlin blue, and then hardened in spirit, and also, if possible, of liver which has been injected through (*a*) the bile-duct, under very low pressure, and (*b*) the hepatic artery, and also sections of small pieces of a fresh liver which has been treated with osmic acid. These injected preparations will show the arrangement of the blood-vessels and bile-capillaries.

Structure.—The liver possesses both a serous and fibrous coat. The former is absent from the posterior border, and from the portal fissure, where the latter, which elsewhere is thin, is most developed.

A strong sheath of areolar tissue (**Glisson's capsule**) surrounds the vessels of the organ as they ramify in it, and, at the transverse fissure, becomes continuous with its fibrous coat.

The **liver substance** proper consists of lobules, which are closely packed polyhedral masses more or less distinct, arranged around the sides of the branches (*sublobular*) of the hepatic veins, and connected to them by minute veins which begin in the centre of the lobules (*intralobular veins*).

Each **lobule** consists of a mass of compressed spheroidal or polyhedral nucleated and nucleolated cells, measuring from $\frac{1}{1000}$th to $\frac{1}{800}$th of an inch in diameter, often containing oil-globules. Surrounding the lobules is a variable amount of fine connective-tissue, in which is contained a minute branch (*interlobular*) of the portal vein, a branch of the hepatic artery and of the hepatic duct, together with minute lymphatic vessels covering them. The lobules are distinct when the interlobular tissue forms complete septa around them; if the septa are incomplete, as is, for example, the case in human liver, the lobules become confluent. Fine fibrous tissue surrounds the interlobular vein, and a delicate supporting network of flattened, branched corpuscles exists within the lobule between the cells and the blood-capillaries. Between the *columns of cells* run the radicles of the hepatic vein which open into the intralobular vein, and *between the cells* begin the radicles of the hepatic duct. Whether these radicles of the bile capillaries have a definite membrana propria is undetermined. The interlobular **bile-ducts** are endothelial tubes with a large lumen, lined with columnar epithelium. The larger ducts are surrounded with circular unstriped muscle-cells, and have a distinct mucous membrane of loose connective-tissue lined with columnar epithelium, and containing mucous tubular glands. The **lymphatics** of the lobule originate in the spaces around the capillaries of the lobules. The branches of the **hepatic artery** run between the lobules with the interlobular veins; in parts they surround the veins as a plexus; the arterial branches frequently anastomose with each other, and give off capillaries to supply the surrounding connective-tissue and vessels, the bile-ducts receiving numerous branches. The ultimate capillaries enter the lobules, where they form a plexus. The blood from the artery is carried away by a special set of veins which open into the interlobular veins; none of it passes into the intralobular veins.

The structure of the **Gall Bladder** is similar to that of the large hepatic ducts, but the mucous membrane is thicker, and is thrown into folds and villous projections. The muscular coat also is thicker, and is surrounded by connective-tissue and an outer layer of peritoneum.

CHAPTER X.

THE RESPIRATORY TRACT.

THE EPIGLOTTIS.

The human epiglottis, removed as soon as possible after death, should be used. It should be cut into small pieces, and placed in the chromic acid and spirit mixture until it is sufficiently hardened. Transverse and vertical sections should be well stained in hæmatoxylin, and mounted in the ordinary way.

Structure.—The epiglottis consists of a supporting cartilage of the elastic variety, enclosed in a fibrous perichondrium, and covered on both sides with mucous membrane.

The **anterior surface** — *i.e.*, the one towards the tongue—is covered by mucous membrane which hardly differs from that of the pharynx. This membrane consists of fibrous tissue, elevated towards the surface, in the form of rudimentary papillæ, and covered with several layers of squamous epithelium. In it ramify the capillary blood-vessels, and in its meshes are a large number of lymphatic channels. Under the mucous membrane in the less dense fibrous tissue, or submucosa, are a number of tubular mucous glands.

The **posterior surface** is covered by a mucous membrane which is similar in structure to the above, but the epithelial coat is thinner, the strata of cells being less numerous. The papillæ are fewer and less distinct. The proper substance of the mucous membrane appears to be in great part adenoid tissue, which here and there is collected into distinct masses. The glands of the posterior surface are smaller but more numerous than those on the anterior surface. In many places the glands which are situated nearest to the perichondrium are directly continuous through apertures in the cartilage with those on the other side, and not unfrequently the ducts of the glands from one side of the cartilage pass through and open upon the mucous surface of the other. Occasionally the epithelium of the posterior surface is columnar.

THE LARYNX.

The structure of the larynx closely resembles that of the epiglottis on the one hand, and that of the trachea on the other.

The **framework** is hyaline cartilage enclosed in a fibrous sheath, and covered entirely by a mucous membrane. The epithelium covering this membrane is columnar ciliated, of which some of the cells are goblet-cells, except over the upper part of the false vocal cords, the arytenoid cartilages, the true vocal cords, and immediately below, where there are stratified squamous cells. There is a distinct basement membrane under the epithelium.

The **mucosa** is a dense fibrous tissue containing a large quantity of adenoid tissue; it is here and there separated from the submucosa, in which lie the glands, by a thin layer of elastic fibres.

The **submucosa** is scanty near the true vocal cords, and contains no glands; elsewhere it is distinct, and in it, as is generally the case with mucous membranes, the large vessels and nerves split up for the supply of the superficial structure. Taste-goblets are found in the epithelium on the posterior surface of the epiglottis, in that covering the ary-epiglottidean folds, and the inner surface of the arytenoid cartilage, and also on the true vocal cords.

THE TRACHEA AND LUNG.

The lungs of a recently-killed rabbit or cat should be distended through the trachea with $\frac{1}{6}$ per cent. solution of chromic acid; the trachea should be tied; and the whole organ should then be immersed in a large quantity of chromic acid of similar strength. The solution should be changed for one of a $\frac{1}{4}$ per cent. after two days; in a week the lung should be cut in pieces, and removed to methylated spirit.

Before cutting sections it is necessary that the embedding mass shall have thoroughly penetrated into and filled up the interstices of the tissues, and so it is best to place the piece of lung to be embedded in the wax mass when it is quite hot. In some cases it is as well to stain the lung, as a whole, with hæmatoxylin, and pass it through alcohol and oil of cloves before embedding. Unless the interstices are filled up, it is almost impossible to cut thin sections.

To free the cut sections from wax, they should be passed through oil of turpentine before putting them into oil of cloves.

A better method is to soak the lung in gum, and then to cut sec-

tions of it by means of the freezing microtome, afterwards removing the gum by placing the sections in warm water.

Thin sections of lung injected through the pulmonary artery with Berlin blue, and through the trachea with ½ per cent. solution of silver nitrate, should be made and treated in the usual manner. Sections of trachea and the large bronchi should also be prepared.

Structure.—The **Trachea** consists of the following parts :

(*a*) An elastic **framework** of incomplete rings or hoops of hyaline cartilages, 16 to 20 in number ; each presents a curve of rather more than ⅔ of a circle. These rings are held together by a strong fibrous membrane, more or less elastic, which not only occupies the interval between them, but is prolonged over their outer and inner surfaces ; behind, where the cartilage is incomplete, the fibrous membrane is strengthened by a continuous layer of unstriated muscle, chiefly arranged transversely.

(*b*) A **submucous coat** of areolar tissue and fat is also present ; it contains immediately beneath the mucous membrane longitudinal fibres of elastic tissue, which are for the most part collected into bundles. Tubular mucous glands are found in this coat, and also upon and beneath it.

(*c*) A **mucous membrane** containing a large amount of lymphoid tissue ; under the epithelium is a basement membrane of flattened cells, which send up processes to the epithelium. In the deeper parts are many elastic fibres. On the surface are several layers of epithelium, of which the more superficial are columnar and ciliated, often branched below to join the connective-tissue corpuscles. Between the branched ends of these cells are smaller elongated cells, prolonged upwards towards the surface, and downwards to the basement membrane. Beneath these are one or more layers of irregularly-shaped cells (Debove's membrane).

The **Large Bronchi** closely resemble the trachea in structure, but the cartilage forms more complete rings, though it is present in smaller segments, which, in a contracted state of the tube, often overlap, and the mucous membrane is thinner, and contains a very distinct layer of circularly-arranged unstriped muscle.

The tissue of the **Lung** is made up of lobules attached to the minute divisions of the air-tubes, by which they are held together, as well as by blood-vessels and interlobular tissue.

The **lobules**, although adherent, are quite distinct; the structure of each represents that of the entire lung, and consists of a minute **air-tube** with terminating **air-cells**, lined with tesselated epithelium, together with the pulmonary and bronchial blood-vessels, lymphatics, nerves, and areolar tissue. The principal divisions of the bronchi divide, generally dichotomously, into branches running in all directions, which never anastomose, but terminate separately in the lobules. Within the lobules each bronchial tube finally ends in small recesses (air-cells, alveoli, or vesicles), having previously lost its cylindrical form, from being beset with similar air-vesicles on all sides; in this condition the tube becomes what is called an **infundibulum.**

The structure of the air-tubes gradually changes as they become smaller. The cartilages become irregularly-shaped plates of different sizes, scattered over the sides of the tubes, gradually becoming fewer, and finally disappearing before the infundibulum is reached. The fibrous coat extends to the smallest tubes, by degrees becoming simply areolar. The mucous membrane becomes thinner, but retains its former epithelium, the cells becoming very short columnar in the smallest bronchi. The longitudinal elastic bundles are traceable into the smallest tubes. The muscular fibres ultimately form a continuous circular layer inside the cartilaginous plates. The walls of the infundibula consist of:

(*a*) Unstriped muscle, arranged circularly.
(*b*) A network of elastic fibres.
(*c*) Fibrous tissue and connective-tissue cells.
(*d*) A dense meshwork of capillary blood-vessels.
(*e*) Small polyhedral cells and large flattened cell-plates. These plates vary in shape and size according to the amount of distension of the air-vesicles; they are best seen in lungs stained with nitrate of silver.
(*f*) Between the cell-plates *pseudo-stomata* may be found—*i.e.*, larger or smaller circular or angular openings similar to the stomata found in serous membranes; they lead into the lymph-canalicular system of the alveolar wall.

The **blood-vessels** constitute a dense capillary plexus upon the alveolar septa; in the contracted lung the capillaries are very sinuous and close together; whilst in the distended lung they are straighter and further apart. Near the pleura and bronchi the capillaries anastomose with the capillaries of the bronchial artery. The larger arterial and venous branches are situated in the interlobular connective-tissue, which is continuous with their outer coat.

The **lymphatics** are arranged in three systems :

(*a*) The subpleural lymphatics, forming a dense plexus, whose meshes mostly correspond with the outlines of the alveoli.

(*b*) The perivascular lymphatics, whose vessels accompany the branches of the pulmonary artery and vein.

(*c*) The peribronchial lymphatics, remaining in the outer coat of the bronchi, and anastomosing freely with the perivascular lymphatics.

CHAPTER XI.

THE SKIN AND ITS APPENDAGES.

SMALL pieces of skin from various parts—*e.g.*, from palm of hand, fingers, or toes, scalp, scrotum, and general surface, should be hardened either in picric acid or in equal parts of chromic acid (½ per cent.) and of methylated spirit for a week, changing the liquid on the second, fourth, and seventh days, and then removing to spirit until required. Sections may be made in various directions (cutting towards the epidermis is the easiest way), stained, prepared, and mounted in the usual manner.

Double staining with picrocarmin as well as with hæmatoxylin is recommended.

Injected specimens of skin may be prepared by injecting 2 per cent. Berlin blue solution into the main artery of a limb of a dog, or one of the upper extremities of a fœtus.

Structure.—The skin consists of two parts :

(1) The **Epidermis**, or external skin, which is made up of several more or less distinct layers—

(*a*) The most superficial horny layer (**stratum corneum**) varies in thickness, and is composed of layers of flattened epithelium, which show nuclei only after treatment with softening reagents—*e.g.*, caustic potash.

(*b*) The next layer (**stratum lucidum**) is generally homogeneous and thin ; it is composed of closely-packed scales.

(*c*) A layer of granular cells (**stratum granulosum**), flat, spindle-shaped, and nucleated, which stain deeply in logwood.

(*d*) Finally the **Malpighian layer** (rete Malpighii, or rete mucosum), consisting of stratified epithelium, the deepest layers of which are columnar, the next more or less cubical 'ridged' cells, connected together by filaments or prickles, and most superficially are layers of flattened cells.

(2) The **Cutis vera**, or true skin, is made up of dense areolar tissue, in which is found, lying deeply, a good deal of fat; muscular fibres occur in the neighbourhood of hairs; they exist as a distinct layer in the subcutaneous tissue of certain parts—*e.g.*, scrotum, penis, areola of the nipple, etc. In the superficial part of the corium are numerous conical elevations or papillæ, which are received into corresponding pits in the epidermis; they are most developed where sensation is most acute. The subjacent or reticular part of the corium contains hair follicles, with sebaceous glands and sweat-glands.

Nerves and **blood-vessels** are numerous; the former, ending in the Malphigian layer in a delicate network, and supplying certain of the papillæ, form special endings (end bulbs and tactile corpuscles); the latter form near the surface a dense network of capillaries with rounded polygonal meshes.

THE CUTANEOUS GLANDS.

(*a*) **Sweat-glands** are found distributed throughout the skin generally, and are exceedingly numerous. Each gland consists of a long duct, which passes through the skin in a more or less wavy manner, to open on the surface, and a coiled gland proper contained in the subcutaneous tissue. The duct of the gland consists of a narrow tube made up of a homogeneous basement membrane, lined with several layers of small cubical epithelial cells limited internally by an endothelial membrane, which bounds a lumen, generally circular in form. The gland proper is made up of the coils of the duct, differing in number according to its situation. The coils nearest the duct proper differ little in structure from the above, but the remainder of the gland (distal portion) is found to have a single layer of columnar cells lining it, instead of several layers of small cells; the internal limiting membrane is less distinct, and the membrana propria, or basement membrane, is strengthened and made thicker by an internal layer of longitudinal unstriped muscular fibres. The glands in the neighbourhood of the anus are exceedingly large.

(*b*) **Ceruminous glands** are similar in structure to the sweat-glands elsewhere, but the gland proper is throughout like that of the distal part of the sweat-gland, as described above.

(*c*) **Sebaceous glands** are racemose glands which, as a rule, open into the neck of hair follicles. Each gland is composed of a short duct, which branches into several dilated alveoli, which may each be further subdivided. The duct is lined with two or three

layers of small nucleated cells, and each alveolus is lined with smaller cubical nucleated cells. The remainder of the alveolus is filled up with cells increasing in size towards the centre, and filled with fat. These central cells have been produced by the division of the lining cells, and, as they reach the centre of the alveolus, pass into the duct, lose their nuclei, and, discharging their fatty contents externally, shrivel up, and are discharged in the sebaceous secretion.

THE HAIR.

Hairs may be seen in sections of the skin or scalp, especially well in double-stained sections which have been placed in picro-carmine and hæmatoxylin. Single hairs may be examined in any reagent, but best in caustic potash. Transverse sections are made in the ordinary operation of shaving, and may be examined in saline solution.

Structure.—The free extremities of hairs above the skin are pointed, the attached extremities are received into follicles in the corium; between the extremities is the shaft. The follicular end is bulbous, and cased in a compound sheath.

A **hair** is made up of:

(*a*) An external covering of thin scales (cuticle).

(*b*) A cortical substance made of coloured horny matter; and finally

(*c*) The medulla or pith, which is absent in some hairs.

The **bulb** of the hair rests upon and overlies an elevation of the follicle (papilla), which is composed of undeveloped nucleated connective-tissue corpuscles and a few fibres.

The **sheath** of the hair is divided into:

(*a*) The internal, of two layers of large cells, the external layer consisting of transparent oval cells without nuclei, and the other layer of polyhedral nucleated cells.

(*b*) The external, of a variable number of layers of cells becoming more columnar externally.

The **hair follicle** consists of an involution of the cutis vera, forming three layers.

(*a*) The external is very thin, made up of longitudinally arranged connective-tissue bundles, with fusiform nuclei and elastic fibres.

(*b*) The middle is thicker, and made up of transverse undeveloped fibrous tissue, with rod-shaped nuclei.

(*c*) The internal, of a thin, striated transparent membrane of endothelial cells.

To the outside of the follicles thin bundles of unstriped muscular fibre are attached, and into the follicle open the ducts of sebaceous glands, generally one on each side.

THE NAILS.

Sections of a finger-nail *in situ* and of the subjacent bed or matrix should be made, double-stained in picrocarmine and hæmatoxylin, and mounted in the usual way.

Structure.—A nail is composed of flattened epithelial scales, and is equivalent to the superficial or horny layer of the epidermis. The deeper layers of the nails are softer than the more superficial. Underneath the nail are highly vascular papillæ which form the bed or matrix. Posteriorly it is received into a groove in the skin (root). The growth of the nail is effected by constant additions of cells to the root and to the under surface, so that it grows in length and in thickness at the same time.

CHAPTER XII.

THE GENITO-URINARY ORGANS.

THE KIDNEY.

The kidney should be hardened in the same way as the liver. Sections should be made in various directions. Sections of an injected kidney should also be prepared. The best injecting material is either carmine-gelatine or Berlin blue (p. 68).

Structure.—There is a distinct fibro-areolar coat, or **capsule**, thin, firm, smooth, and easily detached.

When the kidney has been cut across in its broadest diameter into two vertical halves, the proper substance of the organ is divided into three regions—the **cortical region**, the **boundary layer**, and the **papillary region**.

The cortex is that lighter part nearest the capsule, whilst the redder portion is the medulla, which is seen to be made up of a number of pyramidal portions, each papilla converging to the interior and towards branches (*calyces*) of the dilated portion of the main duct or **pelvis** of the kidney. Each calyx encloses two or three papillæ. The part of the base of the pyramid towards the cortex, between it and the papillary portion, is called, as above mentioned, the boundary layer. The pyramid itself is called the **pyramid of Malpighi**. The papillary portion appears distinctly and vertically striated in consequence of the vertical direction of both tubules and blood-vessels, of which the kidney is principally made up. The boundary layer is also striated for a similar reason; but the cortex, although containing vertical columns, from the arrangement of some of the tubules (**medullary rays**), no longer contains the blood-vessels arranged in vertical directions, nor are all the tubules straight, many are convoluted, forming the **labyrinth**. From the medullary rays diminishing in thickness from the boundary layer outwards towards the capsule, each presents a

triangle, with its base at the boundary layer. These triangles are called the **pyramids of Ferrein**. The cortical substance separates the pyramids from each other, and encloses them everywhere except at the papillæ; one layer of it, situated immediately beneath the capsule, forms the most superficial part of the organ. The portion of the cortical substance intervening between two pyramids is known as the **columns of Bertini**.

The papillæ are studded with minute openings leading into tubes (**tubuli uriniferi**), through which the urine passes out into a primary division (**infundibulum**) of the pelvis, or dilated part of the duct (**ureter**) of the kidney.

The tubes of the pyramids, as they pass up, divide again and again at very acute angles, until they arrive at the cortical layer, where they become convoluted. Each tube begins in a spherical dilatation (**Malpighian capsule**), enclosing a tuft of minute vessels (**Malpighian tuft**). Arising thus in the cortex, a tube is at first convoluted, and consists of a basement membrane lined and almost filled with granular epithelium; afterwards becoming smaller, it passes straight down the pyramid towards the papilla, and returns again, forming a **looped tube of Henle** lined with squamous epithelium, then again becomes convoluted, and finally joins a branch of a straight tube of the pyramid (**collecting tube**). The collecting tubes are lined with columnar epithelium, and, joining together, form the excretory tubes or ducts of Bellini, which open at the papilla.

A renal tube is made up of the following sections:

(1) The **Malpighian capsule**, lined with squamous epithelium.
(2) The **neck**, a constricted portion joining the capsule, and lined in the same way.
(3) A portion enlarged and convoluted, called the **proximal convoluted tube**, lined with polyhedral or short columnar cells, with a lumen of about one-third of the diameter of the tube. The cells are vertically striated.
(4) The **spiral tube**, which passes downwards, the structure of which is similar to the last-described section.
(5) The constricted portion, called the **descending limb of the loop of Henle**, lined with squamous epithelium.
(6) The **loop of Henle**, lined with squamous epithelium.
(7) The **ascending limb**, which becomes rather suddenly enlarged, lined with striated epithelium.
(8) The **spiral portion of ascending loop** is again somewhat constricted.
(9) The ascending loop again becomes narrower, but is **straight**.

(10) The **irregular tubule** has a very irregular and angular outline, sometimes being three or four times as thick as at others; this is due to the irregularity in the size of the contained epithelium. The cells are striated, angular, and imbricated.

(11) The **intercalated section** (Schweigger-Seidel), or the distal convoluted tube, is similar in structure to the proximal convoluted tube.

(12) and (13) **Curved collecting tubes** are thin tubes, lined with polyhedral cells, or spindle-shaped and flattened.

(14) The **straight collecting tube**, which passes into the boundary layer, and enters

(15) The large collecting tube, or **tube of Bellini**.

(16) The tube of Bellini, having anastomosed with similar tubes, forms the **main tube** of the pyramid which opens into the calyx of the pelvis, with a '*mouth*' at the apex of the papilla.

Blood-vessels.—The blood-supply of the kidney is furnished by the renal artery, which divides into branches which lie between the cortex and the boundary region; smaller vessels pass up and enter the cortex, and pass down to supply the medulla. The vessels of the cortex pass up in the labyrinth between the medullary rays (interlobular) and give off transverse branches, the afferent vessels of the Malpighian tufts; these break up within the capsule into convoluted capillaries, re-uniting into the efferent veins; these again break up into capillaries around the convoluted tubes, to be afterwards collected into small branches of the renal vein. The vessels of the medulla break up in the boundary region, and send off straight vessels between the tubes of the papillary region (*vasa recta*); the vessels decrease in number towards the papilla, as most of them break up into capillaries around the tubules, which capillaries anastomose near the cortex with those of that region. The veins of the papillary region begin simply in the papilla, increasing in size and number as they pass upwards; join with the veins of the cortex to form the main branches of the renal vein which accompany the main branches of the renal artery, and lie between the cortex and medulla, as above mentioned.

A certain amount of interstitial connective-tissue is found supporting the tubes and blood-vessels.

THE URETER.

To prepare the ureter for section-cutting distend it with chromic acid and spirit; leave it for one day in the same mixture; then cut

it into short lengths, and remove to spirit for a week. The cells may be shown by hardening a piece of ureter in bichromate of potash 1 per cent. solution, staining deeply in logwood, and scraping the inside, teasing and mounting in glycerine.

Structure.—Consists of three coats:

(1) An external **fibrous.**

(2) A middle of two layers (circular and longitudinal) of unstriped **muscular** fibres.

(3) An internal or **mucous,** lined by stratified epithelium, the upper cubical cells of which have their under surfaces hollowed out to receive the second layer of pear-shaped cells.

THE BLADDER.

The bladder should be prepared in the same way as the ureter. Double-staining with eosin and hæmatoxylin brings into view the differences in the form of the lining cells.

Structure.—The bladder consists of four coats:

(1) An external or **serous**—is incomplete, as it is only found at the upper and posterior parts.

(2) A **muscular,** consisting of three layers more or less complete—viz., (*a*) external longitudinal, (*b*) circular, (*c*) internal longitudinal.

(3) A **submucous** of connective-tissue.

(4) A **mucous,** lined with stratified epithelium, the upper layer being made up of polyhedral cells, with one, two, or three nuclei, presenting depressions with intervening ridges for the second layer of club-shaped cells; the next layer is made up of more fusiform cells.

THE PROSTATE.

The prostate should be immersed in a $\frac{1}{4}$ per cent. chromic acid for two days, and should then be removed to spirit.

Structure.—In structure the prostate consists of small glands imbedded in an abundance of muscular fibres and connective-tissue.

The glands consist of numerous small saccules, opening into elongated ducts, which unite into a smaller number of excretory ducts. The **acini,** in the upper part of the gland, are small and hemispherical; whilst in the middle and lower parts the tubes are longer and more convoluted. The acini are of two kinds:

(*a*) Lined with a single layer of thin and long columnar cells, each with an oval nucleus in outer part of the wall.

(*b*) Acini resembling the foregoing, but with a second layer of small cortical, polyhedral, or fusiform cells between the membrana propria and the columnar cells.

The **ducts** are lined by a layer of columnar cells, beneath which is a layer of small polyhedral cells.

The tunica adventitia is formed of loose connective-tissue containing fat.

Large **blood-vessels** pass into the interior of the organ to form a broad-meshed capillary system. **Nerve-trunks** and numerous large ganglion cells surround the cortex. Pacinian bodies are also found in the substance of the prostate.

THE VESICULÆ SEMINALES.

The vesiculæ seminales may be prepared either in $\frac{1}{6}$ per cent. chromic acid for seven days, followed by spirit, or by hardening in methylated spirit.

Structure.—(1) There is an **external connective-tissue coat.**

(2) A **middle muscular coat** of three layers, the internal of longitudinal fibres, middle of circular fibres, the external of longitudinal fibres.

(3) A **mucous coat** thrown into rugæ, the epithelium of cylindrical cells provided with striated borders, the deep layer being polyhedral. The mucous membrane contains a few muscular fibres.

Ganglion cells and **nerve plexuses** are numerous in the outer coat. According to Leydig, a number of racemose *glands* are present.

THE VAS DEFERENS.

The vas deferens should be prepared by hardening in a 2 per cent. bichromate of potash solution for fourteen days, after which it should be placed in spirit.

Structure.—Like the vesiculæ seminales it consists of three coats:

(1) An **external**, of connective-tissue, outside which longitudinal fibres of unstriated muscles are often seen.

(2) A **muscular**, two longitudinal layers with an intermediate circular one.

(3) A **mucous**, of connective-tissue and elastic fibres; this layer is often thrown into three or four longitudinal ridges; the epithelium consists of columnar epithelium, ciliated only near the epididymis.

The **nerves** form a plexus in the tunica adventitia.

THE TESTICLE.

Place the testicles, preferably of rat, dog, or cat, after making two or three small cuts in them, in equal parts of chromic acid ($\frac{1}{2}$ per cent.) and methylated spirit. Change three times in a week, and remove to spirit, or inject a 1 per cent. solution of osmic acid into the tunica albuginea, then place in strong spirit for several days, and afterwards for two days in absolute alcohol previous to making sections. Stain with hæmatoxylin or carmine; prepare and mount as usual.

Structure.—The outer coat consists of connective-tissue, the **tunica albuginea**, from which radiate incomplete septa uniting into a thick wedge-shaped body, the corpus Highmori or **mediastinum testes**. The position of this body, however, varies in different animals, or it may even be absent altogether.

In man, dog, cat, or rabbit, the testicle is divided by these septa into lobes, each consisting of small and convoluted but, as a rule, unbranched tubes, the **tubuli seminiferi**. These tubes are composed of a basement membrane of flattened endothelial cells, a single row in small animals, more than one in large animals, within which are a number of cells not arranged in any definite order—the **seminal cells**. The *outer* form a single row. The tubuli seminiferi have a uniform diameter of $\frac{1}{150}$ to $\frac{1}{200}$ inch; they commence in free closed extremities or in anastomosing arches, and unite to form the vasa recta. In transverse section the seminal tubules are seen to have a relatively large lumen surrounded by cells. In the testicle of the adult animal these cells are in several layers, the most external being large, polyhedral, and transparent, with some of their nuclei in a condition of karyo-kinesis, and more internally two or more layers of cells, more loosely connected together, more rounded, and somewhat smaller. The cells forming the intermediate layer have nuclei undergoing active division, and consequently, by their multiplication, the inner or daughter cells of small size are formed, termed **spermatoblasts**, from which the **spermatozoa** are developed. The changes which occur in these cells after their formation arise as follows: They become pear-shaped, with the nucleus at the thinner end, flat, and homogeneous, they then elongate and form young spermatozoa, which collect together into fan-shaped groups, with the wide edge of the fan towards the lumen, being massed by means of a granular substance. The nucleus of the cell becoming the head of the spermatozoon, the remainder of the protoplasm is used to produce the rod-shaped middle piece, from which grows out the long filamentous tail. By the solution of the cement substance the spermatozoa

become free in the lumen of the tube. In the interstitial connective-tissue between the tubuli seminiferi are a number of connective-tissue corpuscles.

The **vasa recta**, about twenty in number, are $\frac{1}{90}$ to $\frac{1}{70}$ inch in diameter. They possess very thin walls, and pass upwards and backwards to terminate in the rete vasculosum testis.

The **rete testis** is lined with pavement epithelium, and opens into twelve to twenty **vasa efferentia**, forming the coni vasculosi.

The **coni vasculosi** are $\frac{1}{50}$ inch in diameter, and open into the canal at the epididymis.

The **Epididymis** and **Vasa Efferentia** contain plain muscular fibres; the lining cells are columnar and ciliated, elongated in the epididymis, shorter in the vasa efferentia.

Remove and examine *spermatozoa* from the fresh glands, and notice (*a*) the head, (*b*) the middle portion, (*c*) the caudal extremity.

The **blood-vessels** surround the convoluted tubules with a long-meshed wide capillary plexus.

The **lymph passages** form an extensive canalicular system.

THE PENIS.

The penis of a human fœtus should be used, if it can be obtained, otherwise that of the cat or dog. It may be injected from the abdominal aorta, after ligature of the external iliac arteries, and should then be hardened in $\frac{1}{4}$ per cent. chromic acid for a fortnight. Sections should be made in various parts.

Structure.—(*a*) The **urethra** is lined by stratified pavement epithelium in the lower part of the prostatic and membranous portions; in the upper half the epithelium is of the stratified transitional variety; in front of the bulb the epithelium becomes columnar, whilst the fossa navicularis is again lined with stratified pavement epithelium. The **mucous membrane** consists chiefly of fibrous connective-tissue, intermixed with which are many elastic fibres. It is surrounded by muscular tissue of the unstriped variety. In the membranous portion many large veins run amongst the bundles of muscular tissue. Many mucous glands are present.

(*b*) The **corpora cavernosa** consists of a matrix, chiefly of unstriped muscle-fibres, intermixed with which is a little connective-tissue and a few elastic fibres. The matrix is arranged in bundles, and separates the very large **venous sinuses**, which constitute the greater part of the substance of each corpus cavernosum. The sinuses anastomose with each other to form plexuses, and each is lined by a single layer of flattened endothelial plates. The arteries run in the muscular trabeculæ.

(*c*) The **corpus spongiosum urethræ** consists of an inner portion or plexus of longitudinal veins, and of an outer or really cavernous portion identical in structure with that which has just been described. The **lymphatics** of the penis are very numerous.

The **Nerves** form a dense subepithelial plexus.

Cowper's glands resemble the sublingual gland; they are large compound tubular mucous glands.

THE OVARY.

The ovaries of a cat or rabbit are placed, with as little handling as possible, in a mixture of equal parts of spirit, and $\frac{1}{2}$ per cent. chromic acid solution for two or three days, and afterwards in spirit. The sections should be stained with hæmatoxylin or carmine.

Structure.—The ovary consists of an encapsuled stroma and embedded Graafian follicles.

The **outer coat** or capsule consists of low columnar epithelium cells, beneath which is a firm layer of fibrous tissue.

The **stroma** is made up of fibrous tissue and elastic fibres containing blood-vessels, and in the deeper portion muscular fibres. The cortical portion contains a large number of closely-set vesicles, $\frac{1}{100}$ inch in diameter. Each vesicle, or primordial ovum is surrounded by a corona of small nucleated cells. Below this layer of vesicles are more advanced ova, the deepest being the most mature.

The **Graafian follicle**, $\frac{1}{20}$ to $\frac{1}{6}$ inch in diameter, contains a ripe ovum, and is surrounded by fibrous tissue, and by the **tunica vasculosa**, more internally by the **tunica granulosa**, consisting of several layers of granular prismatic cells. In a thickened portion of the tunica granulosa (**discus proligerus**), the ovum is embedded on the inner surface and to one side of the Graafian follicle. The tunica granulosa is separated from the discus proligerus, except at their point of union, by a space containing a clear albuminous fluid.

The **ovum**, $\frac{1}{120}$ inch in diameter, consists:

(*a*) Of an external, firm, transparent membrane, which is finely striated radially (vitelline membrane, or **zona pellucida**);

(*b*) Of a mass of granular protoplasm (**vitellus**, or yolk);

(*c*) Of a small clear vesicle, $\frac{1}{700}$ inch in diameter (**germinal vesicle**), embedded in the vitellus, and which encloses

(*d*) A dark granular spot (**germinal spot**, or macula germinativa), $\frac{1}{3000}$ inch.

The **Corpus Luteum** is a Graafian follicle which has discharged its ovum; it is filled with a reddish-yellow mass of elongated cells, the

colour being due to the formation of pigment, which, however, is not derived from the slight hæmorrhage which takes place on the escape of the ovum.

THE UTERUS.

The uterus of a cat or rabbit should be distended through the vagina with a mixture of equal parts of ½ per cent. chromic acid solution and strong spirit. The openings into the organ should be tied, and the organ should be removed to a bottle containing the same mixture. The solution should be changed at the end of twenty-four hours and the uterus laid open.

Structure.—The **external serous coat** is derived from the peritoneum.

The **muscular coat** is intermixed with fibro-areolar tissue, blood-vessels, lymphatics, and some veins. The muscle is arranged in three layers:

(*a*) The external longitudinal, the weakest coat.
(*b*) Transverse fibres, forming the strongest layers.
(*c*) Oblique fibres, which become annular to form the sphincter uteri.

The cells constituting the muscular layers are fusiform, with long tapering extremities; the nucleus is always single.

The **mucous membrane** is smooth in the fundus and body of the organ; it is raised into transverse folds in the upper part of the cervix; and forms papillæ in the terminal portion of the cervix. It is lined with columnar ciliated epithelium. The glands are tubular, often spiral, sometimes slightly branched. They are found in the fundus and body, and are lined with ciliated epithelium. Small closed sacs (ovula Nabothi) are also distributed regularly over the mucous membrane.

The **Blood-Vessels** are large and numerous; the *lymphatics* form large plexuses in the peripheral layers of the pregnant uterus; the **Nerves** are medullated and non-medullated, a few ganglion cells being also present.

THE FALLOPIAN TUBES

Are prepared in the same way as the uterus.

Structure.—(1) An **external serous coat**, rich in vessels and in connective-tissue.

(2) A longitudinal and a thicker circular coat of unstriated **muscle**.

(3) A **mucous membrane** thrown into longitudinal rugæ, and lined with columnar ciliated epithelium; no glands are present, and, as yet, no nerves have been detected. The mucous membrane contains a layer of muscularis mucosæ.

THE MAMMARY GLANDS.

The gland of a bitch, cut into small pieces, is placed in a solution of equal parts of spirit and ½ per cent. chromic acid solution for two days, afterwards in weak and strong spirit. It should be stained in hæmatoxylin. Double staining may also be used. Sections should also be cut of pieces of a gland in process of secretion which have been placed fresh in osmic acid.

Structure.—The mammary gland consists of a number of individual racemose glands united by intervening areolar tissue.

The **lobes** thus formed have a considerable quantity of adipose tissue between them, whilst the blood-vessels and the small medullated nerves run in the connective-tissue stroma.

The racemose glands open by means of ducts, the **lactiferous ducts**, which unite together until fifteen to twenty excretory canals are formed.

The **galactophorous ducts**, which converge towards the nipple. Near the nipple the galactophorous ducts become dilated to form sinuses, but they undergo constriction again before opening to the exterior.

The **gland vesicles** consist of a membrana propria with flattened stellate cells, lined by low columnar epithelium. The vesicles are filled with fat-globules; and if the oil be extracted by immersion of the gland in ether, casein remains behind. The terminal vesicles are at first simple, but as the gland develops they produce buds.

The **ducts** consist of areolar tissue with a circular and longitudinal layer of elastic fibres; they are lined with low cylindrical epithelium, which becomes flattened near the nipple. Near the nipple also, and beneath the areola, unstriated muscular fibres are found.

The **Blood-Vessels** form a dense capillary network around the alveoli, forming a continuous system for each lobule.

THE PLACENTA.

Preparation.—Take the uterus from a pregnant cat, rabbit, or guinea-pig, shortly before the time of its delivery. Lay open the uterus carefully, and place it with its contents in a large quantity of Müller's fluid. Change the Müller's fluid on the second, fourth, and sixth days, and then allow it to remain for a fortnight. At the expiration of this time, pour off the Müller's fluid, wash, and afterwards transfer to methylated spirit, which should frequently be renewed.

Structure.—On making a section of the uterine wall at the point

where the placenta is attached, it will be seen that the wall of the uterus is greatly thickened, and that the glands are dilated and elongated. At the point where the chorion is attached to the decidua serotina, the glands have become flattened, as if by pressure, into a compact layer. The bulk of the section, however, is made up of the chorionic villi, whose branchings are so extremely complex that portions of the villi are cut across in every plane. These villi lie in the space between the chorion and the decidua serotina, whilst they are so extensive as almost entirely to obliterate this space. Each villus is covered with a layer of cubical epithelium, and each contains a capillary derived from the umbilical vessels of the fœtus. The villi are separated by spaces which communicate freely with each other, and which in the natural condition contain blood derived from the mother. The spaces between the villi often contain large blood-vessels with well-defined walls.

CHAPTER XIII.

THE DUCTLESS GLANDS.

THE THYROID GLAND.

Preparation.—Specimens should be prepared by immersion of the human thyroid gland for twenty-four hours in a mixture of spirit and water, then in strong spirit, till the tissue is sufficiently hard. It may also be hardened by allowing it to remain for a month in Müller's fluid, or in ¼ per cent. chromic acid for a fortnight. It should be stained in hæmatoxylin.

Structure.—The gland is enclosed by a thin transparent layer of dense areolar tissue, free from fat, containing elastic fibres. This **connective-tissue framework** traverses the interior of the organ in the form of strong trabeculæ; it encloses rounded or oblong cavities, the **vesicles**. The vesicles consist of a thin hyaline membrane lined by a single row of low cylindrical cells. The cavities of the vesicles are filled with a coagulable fluid, or more frequently with a colloidal substance. The colloidal substance increases with age, and the cavities appear to coalesce.

In the interstitial connective-tissue is a round-meshed **capillary plexus**, and a large number of **Lymphatics**. The **Nerves** adhere closely to the vessels.

THE THYMUS GLAND.

Preparation.—As for the thyroid gland. Some sections of the fresh gland should be teased in saline solution in order that the concentric corpuscles may be minutely examined.

Structure.—A **capsule** of thin areolar tissue which sends down processes dividing the gland into lobules. The outer surface of the organ is covered with a layer of flattened cells.

Each **lobe** is made up of a number of polyhedral lobules, connected by delicate areolar tissue, which are in turn composed of small **follicles**. The follicles are composed of adenoid tissue or retiform tissue, the meshes of which are filled up with lymphoid

corpuscles. The follicles are therefore comparable with the spleen, tonsils, lymphatic glands, and Peyer's patches.

Scattered in the adenoid tissue are the concentric **corpuscles of Hassall**, composed of concentrically-arranged nucleated protoplasmic cells, with high refractive index. Of these there are two kinds—one, the smaller, simple; the other, the larger, compound. The **arteries** radiate from the centre of the gland. The **lymphatics** are large. The **nerves** are very minute, the terminations have not been traced.

THE PITUITARY BODY

Should be prepared in the same way as the thyroid.

Structure.—The pituitary body consists of **two lobes**—a **small posterior one**, consisting of gray nerve-tissue; an **anterior larger one**, resembling the thyroid in structure. A **canal**, lined with flattened or with ciliated epithelium, passes through the anterior lobe; it is connected with the infundibulum. The **gland spaces** are oval, nearly round at the periphery, spherical towards the centre of the organ; they are filled with granules and nucleated cells. The vesicles are enclosed by connective-tissue, rich in capillaries.

THE PINEAL GLAND

Should be prepared by hardening in alcohol, or by maceration in Müller's fluid; or, better still, by means of osmic acid.

Structure.—A central cavity lined with ciliated epithelium. The glandular substance is divisible into:

(*a*) An **outer cortical layer**, analogous in structure to the pituitary body; and

(*b*) An **inner central layer**, wholly nervous.

The cortical layer consists of a number of closed follicles, containing:

(*a*) Cells of variable shape, rounded, elongated, or stellate.

(*b*) Fusiform cells.

There is also present a gritty matter, the acervulus cerebri, consisting of round particles aggregated into small masses.

The central substance consists of white and gray matter.

The **blood-vessels** are small, and form a very delicate capillary plexus.

THE SUPRA-RENAL CAPSULE.

May be hardened in bichromate of potash, 2 per cent., for a fortnight, in Müller's fluid for a month, or in osmic acid four hours; in each case complete the hardening in spirit. The glands from the

guinea-pig, rabbit, and other animals, should be used and compared with the human supra-renal.

Structure.—An outer **sheath of connective-tissue** sends in prolongations, and forms the framework of the gland.

The **cortical portion**, divided into:

(*a*) An external layer of closed vesicles, the **zona glomerulosa.** The vesicles contain a finely granular grayish substance, no fat-globules, but generally a few small cells.

(*b*) A layer of cells arranged radially, the **zona fasciculata.** The substance of this layer is broken up into cylinders, each of which is surrounded by the connective-tissue framework. The cylinders thus produced are of three kinds—one containing an opaque, resistant, highly refracting mass (probably of a fatty nature); frequently a large number of nuclei are present; the individual cells can only be made out with difficulty. The second variety of cylinders is of a brownish colour, containing finely granular cells, in which are fat-globules. The third variety consists of gray cylinders, containing a number of cells whose nuclei are filled with a large number of fat granules.

(*c*) The third layer of the cortical portion is the **zona reticularis.** This layer is apparently formed by the breaking up of the cylinders, the elements being dispersed and isolated. The cells are finely granular, and have no deposit of fat in their interior; but in some specimens fat may be present as well as certain large yellow granules, which may be called pigment granules.

The **medullary substance** consists of closed vesicles; of elements of the cortical substance; of numerous blood-vessels; and of an abundance of nervous elements. The cells are poor in fat, and occasionally branched; the nerves run through the cortical substance, and anastomose over the medullary portion.

Blood-Vessels.—The cortical portion is supplied with a rich plexus of capillaries, whose meshes are polyhedral in the outer and middle zones, and more elongated in the zona fasciculata. The medulla is supplied with a very rich plexus of wide capillaries. In all parts the blood-vessels are embedded in the trabeculæ.

Lymph spaces and sinuses, best seen between the cells of the zona fasciculata, but existing in other parts, occupy the intercellular spaces and lacunæ; the efferent lymphatics provided with valves lie in the capsule and in the connective-tissue around the central veins.

CHAPTER XIV.

THE LYMPHATIC SYSTEM.

VESSELS.

MAKE sections of a **thoracic duct** which has been hardened in bichromate of potash, and subsequently in spirit, stain with logwood, and mount in Canada balsam.

Structure.—Lymphatic trunks, such as the thoracic duct and the lymphatics leading to the mesenteric glands, have nearly the same structure as veins, like them consisting of three coats. They are provided with valves, especially at their subdivisions. The endothelial cells lining them are elongated.

To demonstrate the structure of **lymphatic capillaries**, the epithelium covering the central tendon of the diaphragm of a rabbit or guinea-pig, which has been bled to death, must first of all be roughly pencilled off with a camel's-hair brush and distilled water, the tendon should then be stained with nitrate of silver, and mounted in glycerine.

Structure.—Lymphatic capillaries consist of a single layer of sinuous endothelial cells, united together by intercellular substance so as to form a membrane.

GLANDS.

Preparation.—Thin sections of a lymphatic gland from the mesentery of a cat, dog or bullock which has been previously hardened in dilute spirit, Müller's fluid, or in bichromate of potash, should be stained with logwood, and shaken in a test-tube half full of water for thirty minutes or more. They are then to be prepared and mounted in balsam in the ordinary manner.

Structure.—Each lymphatic gland is surrounded by a **capsule**, which consists of connective-tissue intermingled with unstriped muscular fibres. From the capsule are given off a number of **trabeculæ**, which give support to the blood-vessels, and pass into the interior of the gland, so as to divide it into a number of compartments or alveoli, which contain the adenoid tissue or proper tissue of the gland.

The **stroma** of adenoid tissue is arranged in the form of follicles in the cortex, and of rounded cords in the medulla. Between the walls of the alveoli and the proper tissue of the gland are a number of spaces lined by endothelium—the so-called **sinuses** of a lymphatic gland. These sinuses are continuous on the one hand with the afferent vessels, and on the other with the efferent vessels; they contain adenoid tissue, but it has a coarser network than elsewhere.

THE SPLEEN.

Preparation.—Small pieces of fresh spleen are hardened in 2 per cent. solution of bichromate of potash, and subsequently in spirit, till they are fit for making sections. Specimens of spleen, injected through the splenic artery with carmine-gelatine, should also be made and examined.

Structure.—The spleen possesses two coats, a serous and a fibrous.

The **serous coat** is derived from the peritoneum, and covers the organ almost completely.

The **fibrous coat**, or tunica propria, is composed of connective-tissue, which in some animals is intermingled with a large proportion of unstriped muscular fibres. From its inner surface processes or trabeculæ pass into the interior of the organ, and interlace freely, so as to form the **trabecular framework** of the spleen. At the hilum the capsule passes in with the blood-vessels, for which it forms sheaths, which become connected with the trabeculæ above described. The interstices between these trabeculæ contain the proper tissue of the spleen, or **spleen pulp**. The spleen pulp is composed of an adenoid reticulum, forming meshes, with endothelial plates attached; into the meshwork thus formed the small arteries pour their blood, and with these meshes, by their widening out, and by the arrangement of the endothelial plates into a distinct lining, the veins are continuous. The spleen pulp contains red blood corpuscles in all stages of decay and renovation, a large number of colourless corpuscles and blood pigment. The small arteries which pass off almost at right angles from the branches within the trabeculæ, into the spleen pulp, exchange their outer coat of connective-tissue for one of adenoid tissue. This adenoid sheath, which takes the place, as it were, of the lymphatic vessels which surround the arteries as long as they lie within the trabeculæ, forms distinct cords. On section they appear like lymphoid follicles, with arteries occupying some position in immediate relation to them. The cords are not everywhere of the same diameter, but increase here, and diminish there; they are highly vascular when injected. To these circular masses, as seen on section, the term **Malpighian corpuscles** has been applied.

CHAPTER XV.

THE SPECIAL SENSES.

THE EAR.

Preparation.—Remove the lower jaw from a recently-killed guinea-pig in order to expose the auditory bulla. Carefully break open the bulla with a pair of strong scissors after removing the soft parts from it, and look for the cochlea. Remove the cochlea by chipping away the surrounding bone, and immerse it at once in the chromic and hydrochloric acid mixture, or in a saturated solution of picric acid to which some drops of hydrochloric acid have been added, until the bone is soft enough to be cut with ease. Place it next in weak spirit, which should be changed several times. Finally, put the cochlea into absolute alcohol for twenty-four hours before imbedding it. The preparation should be stained in picrocarmine, dehydrated, cleared, saturated with paraffin, and cut with a heavy microtome on a glass plate, or by the ribbon method with the rocking microtome.

Structure.—The **cochlea** is a gradually tapering spiral tube, winding round a central column, the **modiolus**. It is divided along its whole extent by a spiral lamina, which projects from the modiolus, into two main portions—the **scala tympani** and the **scala vestibuli**.

The **spiral lamina** is partially osseous and partially membranous. The membranous portion, the **basilar membrane**, is connected to the outer wall of the cochlea by its union with the *spiral ligament*, which is a projection inwards of the periosteum and subperiosteal tissue of the cochlea.

The **scala vestibuli** is subdivided into **scala vestibuli proper**, and **ductus cochleæ** or **scala media**, by the membrane of Reissner, which passes from the spiral lamina to join the lining periosteum.

The **membrane of Reissner** is composed of a delicate membrana propria, continuous with the periosteum covering the scala vestibuli.

It is lined with a layer of flattened endothelium on the face turned towards the scala vestibuli; whilst that bounding the ductus cochleæ is provided with a single layer of polyhedral cells.

The **periosteum** consists of ordinary connective tissue, thickened here and there by retiform tissue.

The **spiral ligament**, to which the basilar membrane is attached, consists of periosteum thickened by retiform tissue, the cells being elongated, and radiating from the attachment of the basilar membrane. At this point there is generally a large blood-vessel; whilst between the spiral ligament and the membrane of Reissner the periosteum contains pigment-cells and a number of blood-vessels.

The **floor of the ductus cochleæ** is formed of a narrow portion of the spiral lamina, and of the basilar membrane. This portion terminates in a border, which is C-shaped when seen in section, the lower limb of the C being prolonged and tapering. This limb is the end of the osseous lamina; it is covered by a thin membrane. The upper portion of the C is the *limbus* of the spiral lamina, whilst the bay of the C is called the *spiral groove*.

The **limbus** has a jagged edge, as it is raised into a number of tooth-like projections.

The **organ of Corti** forms a portion of the epithelium covering the basilar membrane; it consists of an outer and inner set of stiff rod-like bodies. The feet of the rods rest upon the basilar membrane, whilst they incline towards each other until they meet at their heads. By the meeting of the rods an arch is formed over the basilar membrane; it is filled with endolymph. On the inner side of the inner rods, and the outer side of the outer rods, are epithelial cells with short hair-like prolongations, the **inner and outer hair-cells**; the outer cells are more numerous and more elongated than the inner cells. The hair-like prolongations of the outer hair-cells project through rings which surround the tops of the cells, and which are bounded by minute fiddle-shaped cuticular structures—the **phalanges**. A *reticular membrane* is thus formed, which covers this part of the organ of Corti. On either side of the two sets of hair-cells the epithelium passes continuously into the simple layer of cubical cells, which is found in the spiral groove, and covering the outermost part of the basilar membrane. The whole organ of Corti is also covered by a thick and highly elastic **tectorial membrane**.

The **inner rods** are smaller and more numerous than the outer rods; they may be compared to the upper portion of the human ulna, whilst the **outer rods** resemble the head and neck of a swan.

The concavity of the inner rod receives the rounded portion of the outer rod, which would correspond to the back of a swan's head; whilst the beak of the swan becomes connected to the reticular lamina. Both rods are more slender towards their middle, and expand again, so as to rest by a widened foot upon the basilar membrane; both are longitudinally striated. In the head of the outer rod—and occasionally, also, in the inner rod—is an oval nucleus, staining more deeply than the rest of the cell.

Structure of the Wall of the Membranous Semicircular Canals.—The wall consists, from without inwards, of:

(*a*) An **external fibrous layer**, containing numerous nuclei, blood-vessels, and irregular pigment-cells. This layer is especially developed at the ends of the oval section, where it coalesces with the ligamenta labyrinthi canaliculorum.

(*b*) The **tunica propria**, which presents, after staining, a delicately striated and granular appearance.

(*c*) **Capilliform processes**, which project into the interior of the canal, except at the part where the membranous canal touches the bone.

(*d*) The **epithelium**, a single layer of pavement epithelial cells investing the papillæ; it is continued into the depressions between them.

In the **ampullæ** (*a*) the **fibrous layer** forms a loose meshwork, whilst (*b*) the **tunica propria** is so much thickened as to cause a rounded transverse projection into the cavity—the **crista acustica** or **septum transversum**. (*c*) The **epithelium**, covering the crista acustica, consists (i.) of long cylindrical cells, each with a large nucleus; these cells support the other nervous and epithelial elements, and rest upon the tunica propria; (ii.) fusiform cells which lie between the columnar cells; each cell has a long stiff cilium, the *auditory hair*, and is in direct connection with the ultimate fibrillæ of the auditory nerve. (*d*) The **nerves**, after passing through the tunica propria, form a very delicate plexus in the epithelial layer.

THE NOSE.

Preparation.—To show the **olfactory mucous membrane**, small pieces of the upper turbinal bones from the head of a freshly-killed sheep, dog, or rabbit, should be treated in the chromic acid and hydrochloric acid solution for a week, or in ¼ per cent. bichromate of potash, or in 1 per cent. osmic acid, for forty-eight hours. Sec-

tions may be made through the nasal region of a young guinea-pig's head which has been previously hardened in chromic acid and spirit. The fresh tissue may also be treated according to the chloride of gold method.

To prepare the **Organ of Jacobson** for examination, the anterior part of the head of a young guinea-pig or of a puppy should be placed in a 0·25 per cent. solution of osmic acid for a day, and should then be transferred to absolute alcohol for forty-eight hours. A trace of hydrochloric acid should be added to decalcify the bone, and sections should be cut in a plane parallel to the tip of the nose.

Structure.—In a vertical section through the septum nasi the osseous portion is seen to be invested by **periosteum**, which is immediately covered by a thick layer of elongated tubular glands, some simple, others more complex—the **glands of Bowman**. These glands have an epithelial lining of granular spherical cells at the base; of a more polygonal and less granular form near the excretory duct. The ducts open on the surface between the elements of the external layer. The glands become less numerous and ultimately disappear at the point where the olfactory region passes into the ordinary mucous membrane, being replaced by the mucous glands. The glands are separated from each other by ordinary connective tissue, in the deeper layers of which are **pigment-cells** and free pigment masses, as well as capillaries and ramifications of the olfactory nerves.

The **epithelium**, which covers the olfactory mucous membrane, consists of three layers of cells:

(*a*) A superficial layer of **columnar epithelium** which is not ciliated.

(*b*) Spindle-shaped cells, **sensory cells**, which are arranged between the columnar cells, with their thickest part containing the nucleus on a level with the lower and tapering section of the columnar cells; the end of these cells towards the surface is prolonged outwards, and lies between the columnar cells, and terminates in a small bundle of fine rods which project from the surface; the deeper end of these cells terminates in a fine process, which is connected with a fibrilla of the olfactory nerve-fibres.

(*c*) A deep layer of nucleated **cells of pyramidal shape**, with their bases towards the basement membrane structure of the nasal mucous membrane, and their apices between the cells of the *b* layer.

The external process of the sensory cells is composed of two

substances—an outer, which swells up under the influence of certain reagents, and an internal thread, which remains unaffected. In man and mammalia generally the olfactory cells have no cilia. The *superficial cells* are provided with oval nuclei, and extend through the whole thickness of the epithelial layer. The external portion of these cells is more or less cylindrical, and is striated longitudinally. A row of dots can be distinguished upon the external extremities. The trunks of the *olfactory nerve* run in the glandular layer either obliquely or horizontally. The ultimate fibrils of the nerve pass into the epithelial layer and into the olfactory cells, and are connected with the sensory cells.

The **organ of Jacobson** is bilateral, and consists of a tube lying in the lower or osseous part of the septum narium. It is flattened when seen in transverse section, so as to appear kidney-shaped, and is surrounded by a special investment of hyaline cartilage which is frequently incomplete. This cartilage is known as Jacobson's cartilage. The epithelium lining the tube is similar to that covering the mucous membrane of the nasal cavity, but the epithelium lining the inner or mesial side of the canal is thicker than that on the outer side. The thicker epithelium is called the sensory epithelium. Beneath the epithelium is a fibrous layer, and still deeper are glands and a tissue consisting of a cavernous system of blood-vessels. Numerous branches of the olfactory nerve supply the organ, which also contains a few unstriated muscle-fibres. Nothing is known of its function except that it is accessory to the sense of smell.

THE EYE AND ITS APPENDAGES.

The Eyelids.—*Preparation.*—The eyelids and the lachrymal apparatus of a pig may be used if human material is not available. They should be placed in the usual solution of chromic acid and spirit.

The **skin of the eyelids** consists of an epidermis of flattened cells and of a thin corium. The papillæ of the corium are small, and the subcutaneous tissue is very loose, containing numerous and wide lymphatics; a few groups of fat-cells are present. The sweat-glands are small, and the hairs fine, with small sebaceous glands. At the anterior edge of the free margin of the lids the papillæ become larger, and the hairs are converted into eyelashes. Immediately behind the cilia are the ducts of the **glands of Mohl**, which frequently open into the ducts of the sebaceous glands. The glands of Mohl closely resemble in structure the sweat-glands.

Next to the subcutaneous tissue are bundles of striated fibres of

the **orbicularis muscle**, separated from each other by loose connective tissue, which occasionally contains fat-cells.

The **tarsal plate** is a dense felted mass of fibrous tissue which does not contain any cartilage: its anterior and posterior surfaces are intimately connected by bundles of connective tissue with the skin of the eyelid and with the conjunctiva.

Near the posterior edge of the free margin are the mouths of *the* **Meibomian glands**, arranged in a single row. The ducts are embedded in the tarsal plate, and are in direct connection with single or branched alveoli which are sacculated; the glands resemble sebaceous glands. At the posterior edge of the lid the stratum Malpighii becomes modified, and passes into the conjunctiva. A layer of striped muscle, the **musculus ciliaris Riolani**, intervenes between the mouths of the Meibomian glands and the eyelashes.

The **mucosa** of the conjunctiva palpebræ is a comparatively thick connective-tissue membrane, which generally contains a variable amount of diffuse adenoid tissue.

The **conjunctiva** palpebræ itself consists of one or two layers of small polyhedral cells, upon which is superposed a layer of longer or shorter conical or columnar cells, amongst which some goblet-cells are frequently seen. Small mucous glands are embedded in the tarsal plate in the neighbourhood of the conjunctiva palpebræ.

The **lachrymal gland** is a serous gland, divided into lobes and lobules by prolongations from the connective-tissue capsule. The larger **interlobular ducts** are lined with a layer of thin columnar epithelium cells.

The **intralobular ducts** are also lined with columnar cells, whose external portion is distinctly fibrillated, whilst the inner portion is only slightly striated: the nucleus being situated about the centre of each cell. The intermediate portions of the intralobular ducts, and the parts immediately opening into the alveoli, consist of fine tubes lined with a layer of flattened cell-plates, which are often imbricated.

The **alveoli** are more or less tubular, and are provided with lateral and terminal tubular or saccular branches.

The **membrana propria** consists of branched flattened homogeneous cells, from which septa extend between the cells of the glandular epithelium.

The **glandular epithelial cells** form a single layer of polyhedral, or cubical, granular-looking cells, each provided with a spherical nucleus. The distribution of *blood-vessels* is identical with that of the salivary glands.

Cornea.—The anterior part of a human eye which has been

hardened in 2 per cent. solution of bichromate of potash for a fortnight, and then in spirit, should be used to show the general structure.

To demonstrate the nerves and the connective-tissue cells, the fresh cornea of a frog or rabbit should be cut out, and placed for about an hour and a half in chloride of gold solution ½ per cent., then in slightly acidulated water, after which it should be exposed to the light, according to its intensity, for twenty-four hours or more, and mounted whole (or in sections, in the case of the rabbit's cornea) in glycerine.

In order to stain the ground substance, the cornea of a recently-pithed frog should be pencilled with solid nitrate of silver, well washed in distilled water, and mounted in glycerine.

Structure.—The cornea consists of five layers:

(1) The superficial or **conjunctival layer** is composed of stratified epithelium, of three or four distinct strata of nucleated cells, the lowest of which is columnar.

(2) This layer merges into a thin homogeneous layer (distinct only in the human eye), the **anterior elastic lamina**, or Bowman's membrane, which does not differ from the substance of the cornea proper, except in its greater density, and in the absence of corneal corpuscles.

(3) The **proper substance** of the cornea, made up of alternating layers of fibrous tissue which lie parallel to the surface. These are separated from one another by the ground substance, in which are the cell-spaces, of irregular branched form, which freely communicate with the cell-spaces of their own, as well as of other layers. In the spaces, but not filling them up entirely, are the corneal corpuscles, which are branched cells of various forms. In the chloride of gold cornea, these cells appear as large and branched granular dark-red or black cells with large oblong nuclei containing nucleoli.

(4) Membrane of Descemet, or **posterior elastic lamina**—a firm, structureless, transparent membrane, which is brittle; it is covered by

(5) A layer of polygonal **epithelial cells.**

There are no **blood-vessels** in a healthy cornea.

Nerves enter the proper substance of the cornea, and, after losing their myelin, form a plexus, from which finer branches going forward form the 'sub-epithelial plexus,' from which, again, finer fibrils pass among the epithelial cells, forming the 'intra-epithelial plexus.'

Iris.—The iris from an eye hardened in chromic acid and spirit mixture, together with the ciliary processes, can be cut by means of the freezing microtome; the operation requires much care. The whole iris of a small animal may be mounted and examined in a recent state in saline solution.

Structure.—The iris is principally made up of connective tissue and blood-vessels. This forms the middle layer. In front it is covered by **endothelium**, which may contain pigment, a homogeneous basement membrane intervening. Behind there are similar layers—*i.e.*, a pigmentary layer, *the uvea*, and an intervening basement membrane. Around the inner border is a circular muscle of unstriped fibres, the **sphincter pupillæ**. Under the uvea —*i.e.*, between it and the iris proper—is a thin radiating membrane of muscle cells, or, as some suppose, of elastic tissue, which passes outwards from the sphincter. This is the **dilator pupillæ**. The *blood-vessels* are arranged in dense capillary plexuses in the tissue proper and on the sphincter. The **Nerves** form a plexus near the outer edge, from which pass off medullated fibres, terminating in the dilator pupillæ and in the anterior surface of the iris proper, and also non-medullated nerves to the sphincter.

Crystalline Lens.—(*a*) Harden the eye of a frog, from which the cornea has been removed, in a solution consisting of one part of fuming nitric acid, three parts of water, and one part of glycerine. At the end of twenty-four hours remove it from this solution, and allow it to remain for a day in water. Tease a portion of the lens thus prepared in glycerine, and mount it in Farrant's solution or glycerine.

(*b*) Sections of the lens should be made from eyes which have been hardened for a fortnight in Müller's fluid, and afterwards in weak spirit.

(*c*) The capsular epithelium is best demonstrated by staining the uninjured lens of the frog in nitrate of silver, and afterwards mounting portions of the anterior capsule in glycerine.

Structure.—(*a*) *Of the capsule.* The portion which covers the anterior surface of the lens consists of a thick elastic layer, immediately behind which is a single layer of granular hexagonal epithelium cells, each of which is provided with an oval nucleus. The elastic lamina covering the back of the lens has no such lining epithelium, but is in close contact with the lens-fibres.

(*b*) *The lens* itself is composed of:

(1) The **lens-fibres**, which are elongated bands running from the posterior to the anterior surface; they are broader behind than in front. Each fibre contains a nucleus,

which is more distinct in the peripheral than in the central fibres. Every fibre is hexagonal when seen in transverse sections, and is serrated along its narrow edge, the teeth of one fibre fitting into the notches of its neighbour.

(2) The **interstitial substance** is like that of connective tissue; it is permeated by lymph channels.

Retina.—The posterior part of the eye of a pig (if no fresh human eye can be had) is hardened in Müller's fluid for a week, and then transferred to alcohol; pieces of the retina may then be stained in logwood, and cut. Double-staining with eosin and aniline green, or with aniline rose and aniline green, helps to differentiate the layers.

Another method is to place the posterior part of the eye when fresh in 2 per cent. solution of osmic acid for four hours, then in water for one hour to get rid of the excess of osmic acid, and finally in logwood. The retina, thus treated, should be embedded in cacao-butter, instead of the ordinary wax.

The eye of a cat or dog which has been hardened for a month in Müller's fluid, and subsequently in alcohol, may also be advantageously employed for class purposes. The eye is divided in such a way as to leave the retina, choroid, and sclerotic, for about an eighth of an inch upon either side of the optic nerve. After soaking this piece of tissue in water and then in gum solution in the ordinary manner, sections parallel with the optic nerve are made by means of a freezing microtome. The structure of the retina and of the blind spot are excellently shown, and the method has the further advantage that the retina is less liable to fall to pieces during its manipulation by students, because in the neighbourhood of the blind spot it is more firmly attached to the underlying structures than it is in other parts.

To demonstrate the **retinal rods**, remove the eyes from a recently-killed frog, cut them across, and place the posterior portions for three hours in a 1 per cent. solution of osmic acid; transfer them to a solution of picrocarmine, and allow them to remain in it for a week. Tease the retina, and afterwards mount it in glycerine.

Structure.—The retina consists of eight layers in the following order, from within outwards:

(1) A **layer of nerve-fibres**, which is wanting at the yellow spot: the fibres consist of axis cylinders only; it diminishes in thickness anteriorly.

(2) A **layer of nerve-cells** (ganglionic layer), consisting of cells of a spheroidal or pyriform figure; one process of

each extends into the first layer and is doubtless continuous with it. From the other end of the cell, one or more processes extend outward for a variable distance into the next layer. In the yellow spot there are several layers of these cells; elsewhere, only one layer.

(3) An **inner molecular layer**, a dense network of fibrils which appear as a thick stratum of granular-looking substance not unlike neuroglia. It is formed by anastomosing branches of the ganglion cells, and of the cells of the inner nuclear layer.

(4) An **inner nuclear layer** consists of transparent nucleus-like bodies, of at least four kinds: (*a*) A few connected with the fibres of Müller (to be described below); (*b*) the largest number, like bipolar cells, one pole unbranched, passing inwards, and being connected with a nerve-fibre —the other, thicker and branched, running outwards, is supposed to break up into a plexus in the outer molecular layer; (*c*) unbranched cells found as a complete stratum at the innermost part; (*d*) scattered in the outermost part are rounded cells, of large size, with only one process.

(5) An **outer molecular layer**, thinner than (3), but consisting, as it does, of multipolar cells, with numerous branching processes which anastomose with each other, and with processes from the inner and outer nuclear layers to form a dense meshwork.

(6) An **outer nuclear layer** consists, roughly, of two kinds of corpuscles embedded in a reticular matrix: (*a*) Those connected with the rods are most numerous, and may be considered as dilatations situated in the centre of the fine rod fibres: they have an elliptical striated nucleus, but no nucleolus; (*b*) those connected with the cones are fewer, pear-shaped, not striated, and situated nearer the outer part of the layer in the thicker cone-fibre.

(7) A **layer of rods and cones** is composed of elliptical elongated bodies, *the rods*, and shorter, thicker, club-like bodies, *the cones;* each consists of two parts, inner and outer, of which the outer is transversely striated and smaller, and in the cones tapers to a point, whilst the inner is fibrillated externally, but is homogeneous internally.

(8) The **pigmentary layer** consists of a single stratum of hexagonal pigment-cells, which during life undergo active protoplasmic movements.

The **fibres of Müller** consist of bands, which pass through all the layers of the retina, binding them together; they commence by a broad base, forming by their union the *membrana limitans interna;* and, at the outside of the retina, the *membrana limitans externa.* In the inner nuclear layer they give off processes which contain a clear, oval nucleus. In the outer nuclear layer they break up into fibrils, and partially enclose the rod and cone fibres. These fibres are part of a complex connective-tissue framework, which supports the nervous elements of the retina.

The cellular and nuclear elements of the retina, as well as the nerve fibres, terminate abruptly in front of the eye at the ora serrata. The membrana limitans interna, and Müller's fibres, are continued onwards over the ciliary processes to the iris as the **pars ciliaris retinæ.** The pars ciliaris consists of an internal layer of columnar epithelial cells, each with an oval nucleus, and of an external layer of rounded pigment-cells.

Blood-Vessels.—The arterial and venous branches are situated internally under the layer of nerve-fibres. The capillaries are arranged in plexuses, with large meshes. They occur in the inner molecular layer, one plexus being situated near the inner nuclear layer, the other near the layer of nerve-cells. A plexus also exists in the inner nuclear layer, whilst another lies more superficially in the outer molecular layer.

Ciliary Processes.—The ciliary processes resemble the iris in structure, having a similar connective-tissue basis, containing branched pigment cells, covered by a transparent membrane, the *lamina vitrea,* whilst external to this is the uvea, differing in no way from the uvea of the retina.

The *uvea* is covered by a single layer of transparent columnar cells.

Nicati has recently drawn special attention to these cells under the name of the **uveal gland.** He believes that they secrete the aqueous humour. The ducts of the gland are represented by the canal of Petit, the circular marginal slit of the posterior chamber which separates the iris in front from the crystalline lens and the ciliary processes behind, and the slits connecting these spaces with each other; whilst the channels by which the aqueous humour is absorbed and carried away are lacunæ in the epithelial layer covering the crypts on the anterior surface of the iris—sometimes named the lymphatic lacunæ of Fuchs, or the stomata of Nuck and Cornil—which open into a system of lymphatic channels, that are again continuous with the lymphatic sheaths of the anterior and posterior ciliary veins and of the venæ vorticosæ.

The **ciliary muscle** is connected with the outer part of the ciliary processes, and is made up principally of fibres radiating outwards, but partly of circular bundles of unstriped muscle.

Choroid.—The choroid consists of the following coats:

(*a*) **Lamina fusca**, a loose connective tissue, with corpuscles, both with and without pigment, branched or unbranched; the lamina suprachoroidea is a continuation of the lamina fusca.

(*b*) **Stratum vasculosum**, in which are the large blood-vessels embedded in loose connective tissue.

(*c*) An **elastic layer** containing small arteries and veins, covered on each side by endothelium.

(*d*) **Membrana chorio-capillaris**, which contains the dense capillary meshwork, covered by cells, spindle-shaped and flattened, with or without pigment.

(*e*) **Lamina vitrea** (as above).

(*f*) **Uvea**, or retinal pigment.

Sclerotic.—This coat is made of dense fibrous tissue, the bundles of which in part cross and interlace. Between the bundles are connective-tissue corpuscles, almost precisely similar to those of the cornea. These cells are contained in spaces which form an anastomosing lymph-canalicular system. Non-medullated nerve-fibres are said to exist in a dense plexus in the tissue. Near the ligamentum pectinatum iridis, at the sclero-corneal junction, is a circular canal lined with endothelium; it communicates indirectly with the lymph-spaces mentioned above, and is called the canal of Schlemm.

CHAPTER XVI.

THE HISTOLOGY OF FRESH TISSUES.

I. From a freshly-pithed frog proceed in the following order to prepare the tissues, to show the different elements of their structure:

(1) **Connective-Tissue Corpuscles of Cornea.**—Carefully cut the cornea round at corneo-schleral junction with a sharp pair of scissors. Place it in ½ per cent. gold chloride solution for thirty minutes; then wash, and place in lemon-juice for an hour; wash, scrape away the epithelium which covers it, mount in glycerine, and expose to light. Observe the branching cells stained purple with the gold salt.

(2) **Ground Substance and Lymph-spaces of Cornea.**—Snip off the eyelids and rub the cornea with solid nitrate of silver, then carefully cut it out and expose it to light in a watchglassful of distilled water. When it turns brown mount in glycerine. Observe (*a*) the brown-stained ground substance, and (*b*) the irregularly branched clear lymph-spaces in which the corpuscles lie.

(3) **Endothelium.**—Open the abdomen along the middle line, turn the intestines to one side, observe the kidney lying on the posterior abdominal wall; external to the kidney the peritoneum is separated from the abdominal wall, and forms the covering of the *cisterna lymphatica magna;* pierce this portion of peritoneum with a capillary pipette containing ½ per cent. solution of silver nitrate, and distend the sac with the solution. Expose to light *in situ* for ten minutes, then excise two portions. Examine one in glycerine at once, and the other after staining with log-wood, to demonstrate the nuclei of the endothelial cells.

(4) **White Fibrous Tissue.**—Tear away with forceps one of the toes and tease the tendon in saline solution, examine, irrigate with dilute acetic acid, and note the disappear-

ance of the white fibrous (some fine elastic fibres may then be seen). Take another portion, and tease in glycerine after it has been left lying in alcohol for half an hour. To demonstrate the tendon corpuscles, proceed as directed (p. 47).

(5) **Yellow Elastic Tissue.**—Note the fine membranes which connect the skin with the underlying muscles and form the boundaries of lymph-spaces. Take one, and treat with dilute acetic acid for a few minutes in a watch-glass, mount in glycerine, and examine, observing (*a*) the fine elastic fibres, (*b*) the connective-tissue corpuscles, which are sometimes plainly seen.

(6) **Pigment Corpuscles.** — Irregular branching cells with black pigment may be found in the skin or in the mesentery. Remove a piece of either, and mount in glycerine. (Treatment with dilute acetic acid improves their definition.)

(7) **Adipose Tissue.**—This may be found in the neighbourhood of the kidney. Remove a portion, tease and examine in glycerine.

(8) **Hyaline Cartilage.**—Cut a thin transverse section of the head of the tibia or lower end of the femur, stain in logwood, and mount in glycerine ; or snip off a piece of the thin episternum, scrape away the perichondrium, and treat as before.

II. From another freshly-pithed frog proceed in the following order to prepare the tissues :

(1) **Striped Muscle.**—Place a piece of muscle from the thigh in alcohol for one hour, then tease in glycerine, staining a portion with logwood.

(2) **Unstriped Muscle.**—Distend fully a frog's bladder with gold chloride solution ($\frac{1}{2}$ per cent.) by means of a pipette introduced into the lower part of the intestine which is ligatured above, the external orifice of the cloaca being also pinched up and tied tightly ; tie the neck of the bladder, excise it, and place in more gold chloride solution for half an hour ; then lay it open, scrape away the epithelium, wash thoroughly and soak in a warm saturated solution of tartaric acid, expose to light, and finally mount in glycerine. Observe (*a*) bundles of unstriped muscle, (*b*) non-medullated nerve-fibres.

(3) **Cardiac Muscle.**—Stain a small piece of ventricle deeply in logwood, tease finely, and mount in glycerine.

(4) **Epithelium.**—(The outlines of the epithelial cells are rendered more visible by staining with aniline dyes.)

(*a*) *Ciliated.*—Take a scraping from the roof of the mouth and examine in 0·75 per cent. salt solution; observe the ciliary movement and the shapes of the cells.

(*b*) *Columnar.*—Take a scraping from the carefully washed mucous membrane of the stomach or intestine.

(*c*) *Squamous.*—Scrape the cornea and examine the epithelial cell in saline solution.

(5) **Medullated Nerve-Fibres.**—Take three pieces of sciatic nerve; tease one in glycerine, another in osmic acid, 1 per cent., leaving it until blackened, and place the third in alcohol for ten minutes; then tease it, and place in ether or chloroform for ten minutes longer; then wash with alcohol and stain in logwood. Examine all in glycerine, observing the *nodes of Ranvier* in the second specimen, and the *axis cylinder* and *nucleated sheath* in the third.

(6) **Nerve-Cells.**—These may be demonstrated in one of the following ways:

(*a*) Having turned the intestine aside and cut through the septum cisternæ lymphaticæ, observe the aorta lying between the kidneys with the fine pigmented sympathetic cord on each side of it; remove a portion and tease in glycerine; stain, if desired, in logwood.

(*b*) Lay open the spinal canal, and tease a portion of spinal cord in glycerine.

(*c*) Ganglion-cells may also be found in the Gasserian ganglion, ganglion of vagus, or in the interauricular septum.

Ganglion-Cells in Interauricular Septum.—Render the heart ex-sanguine by gently stroking it towards the aorta and then tying the vena cava with a ligature; then ligature the aorta. Take a capillary pipette with a very fine point, filled with chloride of gold solution $\frac{1}{2}$ per cent., and osmic acid $\frac{1}{4}$ per cent., equal parts, insert the point of a capillary pipette into the sinus venus and distend the heart with the solution, and then withdraw the pipette. After five minutes cut the heart out, and place it in more of the solution for about twenty minutes; lay open the auricles and cut out the septum and mount it in glycerine.

(7) **Retina.**—Open the frog's eye, carefully dissect out the retina, stain with eosin, and tease in glycerine (see p. 171).

PART II

PHYSIOLOGICAL CHEMISTRY.

APPARATUS AND CHIEF REAGENTS REQUIRED.

Test-tubes.
Test-tube stand.
Retort stand.
Platinum foil and wire.
Berlin dishes.
Beakers.
Sand bath.
Filter papers and funnels.
Sulphuric, Nitric, Hydrochloric, Acetic, Tannic, Carbolic, Picric, and Boracic acids.
Centigrade thermometer, urinometer.

SOLUTIONS:

Magnesium chloride and sulphate.
Copper sulphate.
Sodium carbonate, sodium chloride, 10 per cent.
Caustic potash, or soda.
Ammonia and ammonium sulphide, and sulphate (solid).
* Millon's reagent. (Mixed mercury nitrate and nitrite?)
Potassium ferrocyanide.
Potassio-mercuric iodide.
Mercuric nitrate and chloride, mercurous nitrate.
Lead acetate.
Calcium chloride.
Litmus, etc., etc.

Alcohol.
Ether.
Lime Water.
Ozonic ether.
Tinc. Guaiaci.
Solid sodium chloride and magnesium sulphate.

HIS division of the book treats of the chief substances found in the animal body; of foods, and the action of the digestive juices upon them; of the secretions and excretions; and of calculi. For the sake of convenience the substances are treated of in the following order: (1) Proteids, or albuminous substances, which occur in the animal tissues and in food; (2) Albuminoids or gelatins; Nitrogenous bodies, other than proteids: (3) Carbo-hydrates; (4) Oil and fats; (5) Healthy urine, and its constituents; (6) Blood, Milk, and Bile: Gall-stones; (7) Digestive fluids; (8) Abnormal urine and Calculi; (9) Examination of organic substances.

* *Preparation of Millon's Reagent.*—Take equal parts by weight of pure mercury and of nitric acid, add the acid to the mercury, place in a ventilated cupboard, and leave until the mercury is dissolved—if necessary, warming slightly. Then add twice its bulk of water. After a time a crystalline white precipitate falls, and the supernatant fluid, which is the reagent to be used, is decanted.

CHAPTER I.

PROTEIDS OR ALBUMINOUS SUBSTANCES.

Proteids or **Albumins** are bodies which are found to be present in all protoplasm, animal and vegetable, of which, indeed, they constitute the chief part. They are highly nitrogenous substances, and contain the elements carbon, hydrogen, oxygen, nitrogen, and sulphur in certain proportions, which may vary slightly (C, from 51·5 to 54·5; H, 6·9 to 7·3; O, 20·9 to 23·5; N, 15·2 to 17; and S, 0·3 to 2). Associated with proteids in protoplasm are found carbo-hydrates and fatty bodies, as well as nitrogenous organic bodies other than proteids, and some salts—*e.g.*, sodium and potassium chlorides. According to some authorities, the salts are in part contained in the proteid molecule.

General Properties.—The animal proteids are amorphous for the most part (with certain doubtful exceptions), although some vegetable proteids have been obtained in crystalline form; some are soluble, some insoluble in water; some are soluble in saline solutions, some insoluble; all are soluble, but with decomposition, in strong acids and alkalies; are insoluble in strong alcohol and in ether. A large number are coagulated on heating. By the action of ferments certain soluble proteids are converted into insoluble forms. Their solutions, for the most part, will not pass through animal membranes, and exercise a left-handed action on polarized light.

General Reactions.—The solutions of proteids, even if dilute, give the following tests:

(i.) They turn yellow, with or without precipitation, on heating with strong nitric acid; the colour of the cooled solution deepens on addition of ammonia (**Xanthoproteic reaction**).

(ii.) They give with **Millon's reagent** a pink precipitate or mere coloration, either directly or on boiling.

(iii.) They give with an excess of caustic soda or potash, and a drop of copper sulphate, a violet or rose coloration (**Biuret reaction**).* If ammonia is used instead of potash the colour produced is blue.

Many of the proteids give, in addition, the following tests:

(iv.) With excess of acetic acid, and potassium ferrocyanide, they give a white precipitate.

(v.) Boiled with excess of acetic acid and a saturated solution of sodium sulphate they give a white precipitate. This test is used to get rid of all traces of proteids, except peptones, from solution.

(vi.) Coagulated albumen, boiled with strong hydrochloric acid, gives a violet red colour.

(vii.) With cane sugar and strong sulphuric acid they give a reddish violet. With glacial acetic acid and strong sulphuric acid, a violet colour (**Adamkiewicz's reaction**).

(viii.) They are precipitated on addition of:
Citric or acetic acid, and picric acid; or,
Citric or acetic acid, and sodium tungstate; or,
Citric or acetic acid, and potassio-mercuric iodide.

VARIETIES OF PROTEIDS.

Proteids are divided into classes, chiefly on the basis of their solubilities in various reagents. Each class, however, if it contain more than one substance, may often be distinguished by other properties common to its members.

(1) *Native-Albumins.*—Soluble in water and in saline solutions; both dilute and saturated, but not in a saturated solution of ammonium sulphate; coagulated—*i.e.*, turned into coagulated proteid on heating.

(2) *Derived-Albumins.*—Soluble in acids or alkalies; insoluble in saline solutions and in water; not coagulated on heating.

(3) *Globulins.*—Soluble in strong or in weak saline solutions; insoluble in water; soluble in dilute acids and alkalies; coagulated on heating.

(4) *Proteoses.* — Intermediate products in the formation of peptones from other proteids. They are not coagulated by heat; are precipitated but not coagulated by alcohol; give a rose-coloured biuret reaction; are precipitated by nitric acid, the precipitate being dissolved on heating, and

* See p. 207. This reaction is not characteristic of proteids, as similar colours are given with Biuret (p. 207), cyanuric acid, uric acid, xanthine, hypoxanthine, sarcosine, hydrocyanic acid.

reappearing on cooling; some are soluble and some insoluble in water; some are precipitated by magnesium sulphate; and all are precipitated by ammonium sulphate.

(5) *Peptones.*—Soluble in water, saline solutions, acids, or alkalies; not coagulated on heating; not precipitated by ammonium sulphate.

(6) *Coagulated Proteids* are divided into two classes, viz., heat-coagulated proteids and ferment-coagulated proteids. The former are soluble only by the action of the gastric and pancreatic ferments; whilst the latter, comprising Fibrin, Myosin, and Casein, are less insoluble.

CLASS I.

NATIVE-ALBUMINS.*

(A) *Egg-Albumin.*
(B) *Serum-Albumin.*

(A) **Egg-Albumin**:

Preparation.—The white of an egg should be cut up into pieces with a pair of scissors, shaken up with four or five times its bulk of water in a flask, and filtered through muslin.

Properties.—The solution is a transparent, frothy, yellowish fluid, neutral or slightly alkaline in reaction.

With the solution thus made show the following **tests**:

(i.) It gives all of the general proteid reactions.

(ii.) When the solution has evaporated to dryness in a water-bath at a temperature not exceeding 40° C., the egg-albumin is dried up into a yellowish, transparent glassy mass, soluble in water.

(iii.) When heated to a temperature of 70° C., it is coagulated—*i.e.*, changed into a new substance, coagulated proteid, which is quite insoluble in water.

(iv.) The egg-albumin is also **coagulated**, *i.e.*:

(*a*) By the prolonged action of alcohol;

(*b*) By strong mineral acids, especially by nitric acid, also by tannic acid or carbolic acid;

(*c*) By ether, but the coagulum is soluble in caustic soda.

(v.) The solution is **precipitated**—*i.e.*, forms an insoluble compound with the reagent—soluble on removal of the salt

* The native-albumins of muscle, milk, and the glands have received special names, viz., myo-albumin, lact-albumin, and cell-albumin, but their properties show no very marked differences from the chief native-albumins here treated of.

by dialysis, without coagulation, with either mercuric chloride, lead acetate, copper sulphate or silver nitrate, the precipitate being soluble in slight excess of the reagent. The albumin is also completely precipitated by saturation with ammonium sulphate or with sodio-magnesium sulphate.

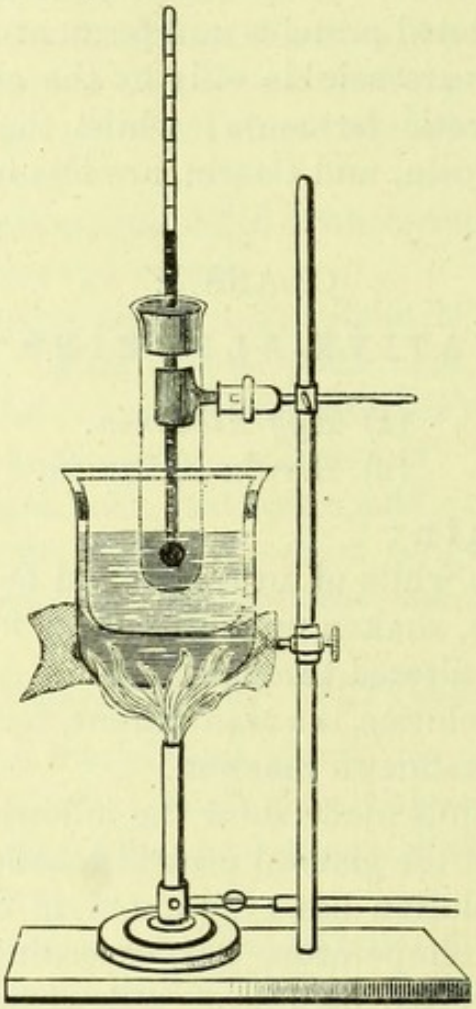

Fig. 65.—A simple apparatus * for investigating the Coagulation Temperature of Proteids.

(vi.) The solution will give *Heller's test*, which is a delicate one for mere traces of the albumin in solution. Pour a little strong nitric acid into a test-tube, and add gradually a dilute solution of egg-albumin; the albumin is precipitated at the point of contact with the acid in the form of a fine white or yellow ring.

(B) **Serum-Albumin**:

Preparation.—Blood serum should be diluted with four times its bulk of water. It should then be neutralized with very dilute acetic acid, and the precipitate which occurs should be removed by filtration.

With the filtered solution show the following **tests**:

* After Gamgee.

(i.) It gives similar reactions to egg-albumin, but differs from it in not being coagulated by ether.

(ii.) It also differs from egg-albumin in not being easily precipitated by hydrochloric acid, and in the precipitate being easily soluble in excess of this acid. Serum-albumin, either in the coagulated or precipitated form, is more soluble in excess of strong acid than egg-albumin.

Dialysis.—Native-albumins are very slightly diffusible. Show this by taking a solution of egg- or serum-albumin and placing it in

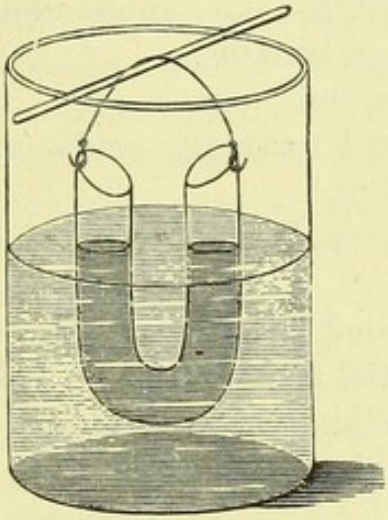

Fig. 66.—A vessel of water with a Sausage Skin Dialyser suspended in it.

a dialyser, and testing the outside liquid from time to time for albumin.

Salt should be mixed with the solution of native-albumin, and tested for in the outside liquid. It is speedily detected; not so the albumin.

CLASS II.

DERIVED-ALBUMINS.

(A) *Acid-Albumin.*
(B) *Alkali-Albumin.*
(C) *Caseinogen.*

(A) Acid-Albumin:

Preparation.—Acid-albumin is made by adding small quantities of dilute acid (of which the best is hydrochloric 0·4 per cent. to 1 per cent.), to either egg- or serum-albumin diluted five to ten times, and keeping the solution at a temperature not higher than 50° C. for not less than half an hour.

Acid-albumin may also be made by dissolving coagulated native-albumin in strong acid, or by dissolving any of the globulins in acids.

With a solution of acid-albumin show the following **tests**:

(i.) It is not coagulated on heating, but is precipitated on *exactly* neutralizing the solution. This may be shown by adding to the acid-albumin solution a little aqueous solution of litmus, and then dropping a weak solution of caustic potash from a burette, until the red colour disappears. The precipitate is the derived-albumin. It is soluble in dilute acids, dilute alkalies and dilute solutions of alkaline carbonates.

(ii.) It gives the proteid tests.

(iii.) It is coagulated by strong acids—*e.g.*, nitric acid—and by strong alcohol.

(iv.) It is insoluble in distilled water, and in neutral saline solutions.

(v.) It is precipitated by saturation with sodium chloride.

(vi.) There is partial coagulation on boiling in lime-water, and further precipitation on addition to the boiled solution of calcium chloride, magnesium sulphate, or sodium chloride.

(B) Alkali-Albumin:

Preparation.—If solutions of native-albumin, or coagulated or other proteid, be treated with dilute or strong fixed alkali, alkali-albumin is produced.

Solid alkali-albumin may be prepared by adding caustic soda or potash, drop by drop, to undiluted egg-albumin, until the whole forms a jelly. This jelly is soluble in dilute alkalies on boiling.

With a solution of alkali-albumin show the following **tests**: A solution of alkali-albumin gives the tests corresponding to those of acid-albumin. It is not coagulated on heating. It is thrown down on neutralizing its solution, except in the presence of alkaline phosphates, in which case the solution must be distinctly acid before a precipitate falls.

To differentiate between Acid- and Alkali-Albumin, the following method is useful:

(1) Alkali-albumin is *not* precipitated on *exact* neutralization, if sodium phosphate has been previously added.

(2) Acid-albumin is precipitated on exact neutralization whether or not sodium phosphate has been previously added.

(c) **Caseinogen**:

Preparation.—Caseinogen may be prepared from milk by the following process: The milk should be diluted with three to four times its volume of water, sufficient dilute acetic acid should then be added to render the solution distinctly acid (not more than a few drops), and the caseinogen, which falls as a precipitate carrying with it the fat, should be filtered off. To purify it, it should be washed with alcohol and then with ether.

Caseinogen may also be prepared by adding to milk an excess of crystallized magnesium sulphate or sodium chloride, which causes it to separate out. The solution is then filtered, and the precipitate collected on the filter is washed with a saturated solution of magnesium sulphate, and the caseinogen is next dissolved from the adhering fat by the addition of distilled water; the solution can afterwards be reprecipitated with acetic acid.

Caseinogen gives much the same tests as alkali-albumin.

It is soluble in dilute acid or alkalies.

It is reprecipitated on neutralization; but if potassium phosphate be present, the solution must be distinctly acid before the caseinogen is deposited.

It differs from alkali-albumin in that it can be converted by a ferment into casein (pp. 192, 224).

CLASS III.

GLOBULINS.

(A) *Globulin.*
(B) *Serum-Globulin.*
(C) *Fibrinogen.*
(D) *Vitellin.*
(E) *Globin.*
(F) *Muscle-Globulins.*

General Properties of Globulins.—They give the general proteid tests.

Are insoluble in water.

Are soluble in dilute saline solutions.

Are soluble in acids and alkalies forming the corresponding derived-albumin.

Most of them are precipitated from their solutions by saturation with solid sodium chloride or magnesium sulphate.

Are coagulated on heating.

(A) **Globulin** or **Crystallin:**

Preparation.—It is obtained from the crystalline lens by rubbing it up with powdered glass, extracting with water or dilute saline solution, and by passing through the extract a stream of carbon dioxide.

Tests.—It differs from other globulins, except vitellin, in not being precipitated by saturation with sodium chloride.

(B) **Serum-Globulin** or **Paraglobulin:**

Preparation.—Paraglobulin may be precipitated (as a bulky flocculent substance) by saturating serum with solid sodium chloride or magnesium sulphate, which can be removed by filtration after standing for some time.

It may also be prepared by diluting blood-serum with ten vols. of water, and passing carbonic acid gas rapidly through it. The fine precipitate is collected on filter, and washed with water containing carbonic acid gas, and also by the addition of very dilute acetic acid to diluted serum.

With a solution of paraglobulin, show the following **tests:**

It is very soluble in dilute saline solutions, from which it is precipitated by carbonic acid gas and dilute acids.

Its solution is coagulated at 70° C.

Even dilute acids and alkalies convert it into acid- or alkali-albumin.

(C) **Fibrinogen:**

Preparation.—Fibrinogen is prepared from hydrocele or other serous transudation by methods similar to those employed in preparing paraglobulin from serum.

Tests.—Its general reactions are similar to those of paraglobulin.

Its solution is coagulated at 52°-55° C.

Its characteristic property is that, under certain conditions, it forms fibrin.

(D) **Vitellin:**

Preparation.—Vitellin is prepared from yolk of egg by washing with ether until all the yellow matter has been removed. The residue is dissolved in 10 per cent. saline solution, filtered, and poured into a large quantity of distilled water. The precipitate which falls is impure vitellin.

It gives the same **tests** as myosin (p. 192), but is not precipitated on saturation with sodium chloride.

It coagulates between 70° and 80° C.

(E) **Globin**:

Is the proteid residue of hæmoglobin.

(F) **Muscle-Globulins**:

Of these there are, according to Halliburton and other authorities, no less than three—viz., myosinogen, paramyosinogen, and myoglobulin. All three exist in the plasma; but the two first go to form muscle-clot, or myosin, the other, myoglobulin, is found in the muscle-serum—muscle-clot, or myosin, will be considered for the sake of convenience.

CLASS IV.

PROTEOSES.

These substances are intermediate products, formed in the digestion of proteids. They are called albumoses, globuloses, vitelloses, caseoses, myosinoses, etc., according to the original proteid from which they are produced. They are ultimately formed into peptones, and are called hemi- or anti-proteoses, according as they form peptone, capable or incapable of being further split up, *e.g.*, into leucin and tyrosin, in the intestine. They may also be formed by heating with water, by dilute mineral acids, or by superheated steam. There are three chief kinds:

(1) **Proto-Albumose.**—Soluble in hot and cold water, and in saline solutions; precipitated by saturation with sodium chloride or magnesium sulphate. It is precipitated by nitric acid, the precipitate being soluble on heating, and reappearing on cooling. It is precipitated by copper sulphate, and gives a rose-colour with the biuret reaction.

(2) **Hetero-Albumose.**—Insoluble in water; soluble in sodium chloride solutions 0·5 to 15 per cent. in the cold, but precipitated at 65° C., the precipitate being soluble in dilute acid or alkali. Precipitated by alcohol, and partially coagulated. Is precipitated on saturation with neutral salts. It is precipitated by copper sulphate, and gives a rose biuret reaction.

(3) **Deutero-Albumose.**—Soluble in cold and hot water, and in hot and cold saline solutions. It is precipitated by saturation with ammonium sulphate, but not with other neutral salts. It gives the nitric acid precipitate when an excess of salt is present. It is not precipitated with copper sulphate, but gives a rose biuret reaction.

For practical work to show albumose reactions, refer to pp. 190, 231, 232.

Exercise.—Take commercial peptone, and dissolve in water; slightly acidify with dilute acetic acid, and saturate with crystallized ammonium sulphate—the precipitate which appears consists of albumoses; separate by filtration—the filtrate contains the peptone. Wash the precipitate with a saturated solution of ammonium sulphate; dissolve it by adding water; slightly acidify with acetic acid; saturate with sodium chloride.

Precipitated.	*Not Precipitated.*
PROTO-ALBUMOSE.	DEUTERO-ALBUMOSE.
Wash the precipitate separated by filtration with sodium chloride solution, dissolve by adding water, and dialyse the solution.	Apply tests as above.

Precipitated.	*Not Precipitated.*
HETERO-ALBUMOSE.	PROTO-ALBUMOSE.
Apply tests as above.	Apply tests as above.

CLASS V.

PEPTONE.

Peptone:

Preparation.—Peptone is formed by the action of the digestive ferments, *pepsin* or *trypsin*, on other proteids, and on gelatine (pp. 231, 232).

Take the filtrate, which results after the separation of albumoses from a solution of commercial peptone by saturation with ammonium sulphate, concentrate and remove by filtration the crystals of ammonium sulphate which separate; add baryta and barium carbonate to get rid of the remaining ammonium sulphate; add dilute sulphuric acid, and remove barium sulphate which is precipitated by filtration; concentrate the filtrate, and add absolute alcohol; remove the precipitate of peptone which forms, and redissolve in water.

With a solution of peptone in water, show the following **tests**:

(i.) It is not coagulated on heating.

(ii.) It is not precipitated by saturation with sodium chloride, or magnesium sulphate, or ammonium sulphate, or by carbonic acid gas.

(iii.) It is not precipitated by boiling with sodium sulphate and acetic acid.

(iv.) It is not precipitated by the addition of a dilute acid or alkali.

(v.) It is precipitated from neutral or slightly acid solutions by:

Mercuric chloride, the precipitate being only partly soluble in excess.
Argentic nitrate.
Lead acetate.
Potassio-mercuric iodide.
Bile salts.
Phosphoro-molybdic acid.
Tannin, the precipitate soluble in dilute acid, not in excess.
Picric acid (saturated solution), the precipitate disappears on heating and partly returns on cooling.
Absolute alcohol (*not* with dilute spirit). N.B.—It is not *coagulated*.
Ether.

(vi.) The solution gives:
The *Xanthoproteic reaction* easily, but there is no previous precipitation with the nitric acid.
The *Biuret reaction*, with a mere trace of copper sulphate—but the colour is *rose* instead of *violet*.
With *Millon's* test—not so easily as with native-albumins.
With *Ferrocyanide and acetic acid* there is no precipitate.

(vii.) It *dialyses freely*.—Show this with a simple gut dialyser in the manner indicated (p. 185).

CLASS VI.

COAGULATED PROTEIDS.

Coagulated proteids are formed either by the action of heat or of ferments upon other proteids. They may also be produced by the prolonged action of alcohol.

A.—Heat Coagulated Proteids.

Tests.—They are soluble in strong acids or alkalies; slightly so in dilute.

Are soluble in digestive fluids (gastric and pancreatic).

Are insoluble in saline solution.

B.—Ferment Coagulated Proteids.

(1) **Fibrin** (*Blood-Clot*):

Preparation.—Fibrin can be obtained as a soft, white, fibrous, and very elastic substance by whipping blood with a bundle of

twigs, and washing in a stream of water until all the adhering blood-colouring matter be removed.

Tests.—It differs from all other proteids, in having a filamentous structure. Examine a specimen with the microscope.

It is insoluble in water and in dilute saline solutions, slightly soluble in concentrated saline solutions, soluble on boiling in strong acids and alkalies.

On boiling it is converted into coagulated proteid.

When dissolved in strong saline solution, it gives many of the same reactions as myosin. When dissolved in acids or alkalies, is converted into corresponding derived-albumin.

It gives a blue colour with tincture of guaiacum and ozonic ether.

(2) **Myosin** (*Muscle-Clot*):

Preparation.—Myosin may be prepared from dead muscle by removing all fat, tendon, etc., and washing repeatedly in water until the washing contains no trace of proteids, mincing it and then treating with 10 per cent. solution of sodium chloride, which will dissolve a large portion into a viscid fluid, which filters with difficulty. If the viscid filtrate be dropped little by little into a large quantity of distilled water, a loose white flocculent precipitate of myosin will occur.

Tests.—With a solution of myosin, show that:

(i.) It is soluble in 10 per cent. saline solution.

(ii.) It is coagulated at 60° C. into a coagulated proteid.

(iii.) It is soluble without change in very dilute acids.

(iv.) It is precipitated by picric acid, the precipitate being redissolved on boiling.

(v.) It may give a blue colour with ozonic ether and tincture of guaiacum.

(3) **Casein** (*Milk-Clot*). (*Tyrein.* Foster):

This is the real curd of milk, which is formed by the action of *rennet ferment* (rennine) upon milk. It can also be formed from caseinogen, which has been isolated from milk, in the manner described above (p. 187), and purified. Refer to exercise on milk (p. 224).

CHAPTER II.

GELATINS OR ALBUMINOIDS; NITROGENOUS BODIES OTHER THAN PROTEIDS.

(A) *Gelatin.*
(B) *Mucin.*
(C) *Elastin.*
(D) *Chondrin.*
(E) *Keratin.*

(A) Gelatin:

Preparation.—Gelatin is obtained from bone, teeth, fibrous connective-tissue, tendons, ligaments, etc., by prolonged action of boiling water in a Papin's digester or of dilute acetic acid at a low temperature (15° C.). It is said to exist in the tissues as **collagen**, which is the anhydride of gelatin.

Properties.—One of the formulæ of gelatin which have been given is $C_{102}H_{151}N_{31}O_{29}$, and it is thus derived from collagen ($C_{102}H_{149}N_{31}O_{28}+H_{20}=C_{107}H_{151}N_{31}O_{29}$). It contains more nitrogen and less carbon than proteids. It yields glycin, leucin, various fatty acids, carbonic dioxide, and ammonia on boiling with sulphuric acid and on putrefaction.

It is amorphous, and transparent when dried. It does not dialyse.

It is insoluble in cold water, but swells up to about six times its volume: it dissolves readily on the addition of very dilute acids or alkalies.

It is soluble in hot water, and forms a jelly on cooling, even when only 1 per cent. of gelatin is present. Prolonged boiling in dilute acids, or in water alone, destroys this power of forming a jelly on cooling.

Tests.—A fairly strong solution of gelatin—2 per cent. to 4 per cent.—gives the following reactions:

(*a*) *With proteid tests:*

(i.) *Xanthoproteic test.*—A light yellow colour with no previous precipitate with nitric acid, becoming somewhat darker on the addition of ammonia.

(ii.) *Biuret test.*—A blueish-violet colour.

(iii.) *Millon's test.*—A pink or white precipitate.
(iv.) *Potassium ferrocyanide and acetic acid.*—No reaction.
(v.) Boiling with sodium sulphate and acetic acid.—No reaction.
(vi.) Saturation with ammonium or magnesium sulphate precipitates it.

(*b*) *Special reactions :*

(i.) No precipitate with acetic acid.
(ii.) No precipitate with hydrochloric acid.
(iii.) A white precipitate with tannic acid, not soluble in excess or in dilute acetic acid.
(iv.) A white precipitate with mercuric chloride, unaltered by excess of the reagent.
(v.) A white precipitate with alcohol, ether, or chloroform.
(vi.) A yellowish-white precipitate with picric acid, dissolved on heating and reappearing on cooling.

Bone consists of an organized matrix of connective-tissue, containing a collagen, **ossein**, which yields gelatin on boiling, impregnated with inorganic salts.

The inorganic salts can be removed by digesting bone in hydrochloric acid. The ossein left retains the form of the bone. By long boiling in water, it is converted into a solution of a gelatin.

When bone is heated, the first action is to decompose the organic matter, leaving a deposit of carbon. On further ignition in air, this carbon burns away, and only inorganic salts (principally calcic phosphate) are left.

Take two pieces of bone, calcine one piece, and boil another in dilute hydrochloric acid in a test tube ; test the ash from the one for phosphates, and test the undissolved part of the other, and also the solution in which it has been boiled, for gelatin.

(B) **Mucin :**

Preparation.—Mucin is the characteristic component of mucus ; it is contained in fœtal connective-tissue, tendons, and salivary glands, and in bile. The substances obtained from these different sources are probably somewhat different in composition. They are, however, all glucosides, and can be split up into a proteid, and a carbo-hydrate substance which can reduce copper salts like sugar.

It may be prepared from ox-gall, by acidulation with acetic acid and subsequent filtration, or from ox-gall by precipitation with alcohol, afterwards dissolving in water, and again precipitating by means of acetic acid.

It can also be obtained from mucus by diluting it with water, filtering, treating the insoluble portion with weak caustic alkali, and precipitating the mucus with acetic acid.

Properties.—Mucin has a ropy consistency.

It is precipitated by alcohol and by mineral acids, but is dissolved by excess of the latter.

It is dissolved by alkalies and in lime-water.

It gives the proteid reactions with Millon's reagent and with nitric acid, but not with copper sulphate and caustic potash.

Neither mercuric chloride nor tannic acid gives a precipitate with it (?).

It does not dialyse.

(c) **Elastin** is found in elastic tissue, in the ligamenta subflava, ligamentum nuchæ, etc.

Preparation.—Take the fresh ligamentum nuchæ of an ox, cut it in pieces, and boil in alcohol and ether to remove the fat. Remove the gelatin by boiling for some hours in water. Boil the residue with acetic acid for some time, and remove the acid by boiling in water, then boil with caustic soda until it begins to swell. Remove the alkali, and leave it in cold hydrochloric acid for twenty-four hours, and afterwards wash with cold water.

Properties.—Elastin is insoluble, but swells up both in cold and hot water. Is soluble in strong caustic soda.

It is precipitated by tannic acid; does not gelatinize. Gives the proteid reactions with strong nitric acid and ammonia, and imperfectly with Millon's reagent.

It yields leucin on boiling with strong sulphuric acid.

(d) **Chondrin** is found in cartilage in the form of collagen. Chondrin is said to be a mixture of gelatin and mucin.

Preparation.—By boiling small pieces of cartilage for several hours, and filtering. The opalescent filtrate will form a jelly on cooling. Chondrin is precipitated from the warm filtrate on addition of acetic acid.

Properties.—It is soluble in hot water, and in solutions of neutral salts—*e.g.*, sulphate of sodium, in dilute mineral acids, caustic potash, and soda. Insoluble in cold water, alcohol, and ether. It is precipitated from its solutions by dilute mineral acids (excess redissolves it), by alum, by lead acetate, by silver nitrate, and by chlorine water. On boiling with strong hydrochloric acid, it yields grape-sugar and certain nitrogenous substances. Prolonged boiling in dilute acids, or in water, destroys its power of forming a jelly on cooling.

(e) **Keratin** is contained in hair, hoofs, nails, and horny substances. It can be dissolved by boiling in glacial acetic acid. When boiled with strong sulphuric acid it is decomposed and yields leucin, tyrosin, aspartic acid, and volatile fatty acids, as do proteids.

CHAPTER III.

CARBO-HYDRATES.

CARBO-HYDRATES are bodies composed of six or twelve atoms of carbon with hydrogen and oxygen, the two latter elements being in the proportion to form water.

The chief carbo-hydrates are included in the following list. They are arranged into three classes; the members of each class with which we have to do are printed in ordinary type, the most important of the others in italics.

TABLE OF THE CHIEF CARBO-HYDRATES.

AMYLOSES.	SACCHAROSES OR SUCROSES.	GLUCOSES.
$(C_6H_{10}O_5)$.	$C_{12}H_{22}O_{11}$.	$C_6H_{12}O_6$
Starch. Dextrin. Glycogen. *Inulin.* *Cellulose.* *Gum.*	Saccharose, *or cane sugar.* Lactose. Maltose. *Melitose.* *Melizitose.* *Mycose.*	Dextrose or grape sugar. Lævulose or fruit sugar. Inosit. *Mannitose.* *Galactose.*

Starch ($C_6H_{10}O_5$) is contained in nearly *all plants:*

Starch may be very easily obtained from a *potato*, by grating it into a bowl of water, allowing it to settle, and pouring off the supernatant water; a layer of starch will remain at the bottom of the vessel; washing several times, each time allowing the starch to settle and pouring away the water; when clean, the starch may be dried at a moderate temperature.

Or from *flour*—by tying some flour in a bag and washing it into a vessel under the tap; allowing to settle, and proceeding as in the other case.

Or it may be obtained from many *seeds*, *roots*, *stems*, and *some fruits*, by somewhat similar treatment.

Characters.—It is a soft white powder, having a soapy feel when rubbed between the fingers, composed of granules having an organized structure, with a centric or excentric spot or hilum, about which alternate layers of cellulose and granulose are arranged, consisting of *granulose* (soluble in water) and *cellulose* (insoluble in water); their shape and size vary according to the source whence the starch has been obtained.

With some starch obtained from the potato, from arrowroot, from rice, or from flour, show the following **tests**:

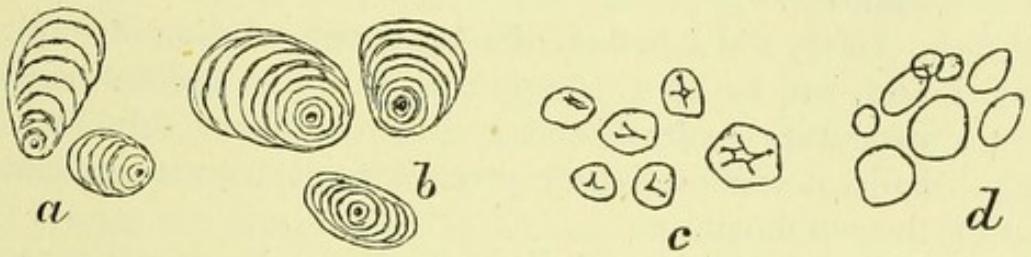

Fig. 67.—Starch Granules.
(*a*) potato; (*b*) arrowroot; (*c*) rice; (*d*) wheat.

(1) Examine some of the dry powder under the microscope ($\frac{1}{4}$ to $\frac{1}{6}$ power), then irrigate the specimen (*a*) with distilled water, note the shape and size of the granules, and that each granule of potato or arrowroot starch shows a more or less excentric hilum, about which are arranged parallel rings. (*b*) With a dilute solution (0·5 to 0·25) of iodine the granules become more or less blue.

(2) The powder is insoluble in cold water, in alcohol, and in ether.

(3) The powder is soluble in hot water, and may be filtered. The hot water causes the granulose to swell up, burst the cellulose coat, and become free, forming soluble starch or **amylin**. The solution is **opalescent**, and, if very strong, sets into a jelly on cooking.

In order to prepare a standard solution of starch—*e.g.*, 1 per cent.—which is useful for the following experiments, 1 grm. of starch should be taken, which should be rubbed up in a mortar with 20 cc. of cold distilled water until thoroughly suspended in the water, then 80 cc. of boiling water should be gradually added with constant stirring. The solution should be filtered.

With the **starch solution** thus made show the following **tests**:

(1) Take 5 cc. of the solution in a test-tube, and add to it 1 to 2 cc. of a standard solution of iodine, an intense **blue** colouration will be produced; warm the test-tube gently, and the colouration will disappear; cool it under the tap, and the colouration will reappear (perhaps not so intensely).

(2) Repeat the experiment, but previously render the starch solution alkaline with potassium or sodium hydrate; the blue colouration with iodine solution will not occur, but on neutralization with dilute sulphuric acid it will do so.

(3) Place 5 cc. of the solution in three test-tubes. To (*a*) add an excess of potassium hydrate and a drop of a solution of copper sulphate, and boil. No reduction of the copper takes place; starch alone has no power to reduce copper-sulphate.

To (*b*) add 1 to 3 cc. of a 1 per cent. solution of sulphuric acid, boil for 10 to 15 minutes, and then add an excess of potassium hydrate and a drop of copper sulphate, and boil; a reddish-yellow precipitate of copper suboxide is thrown down.

To (*c*) add 1 cc. of filtered saliva. Keep at a temperature of 40° C. for 2 to 5 minutes, then add an excess of potassium hydrate and a drop or two of copper sulphate, and boil; a reddish-yellow precipitate of copper suboxide is thrown down.

Starch is converted into sugar by dilute acids and also by saliva, which contains a diastasic ferment, ptyalin (p. 233).

(4) To some of the solution in a test-tube add tannic acid; a yellow precipitate, dissolved on boiling, will be thrown down.

(5) Place some of the solution in a dialyser; in 1 to 2 hours test the outside fluid for starch; there will be no reaction. Starch does not possess the property of passing through animal membranes.

Glycogen:*

Preparation.—Glycogen, which is usually obtained from the livers of animals, is also present to a considerable extent in the muscles of very young animals. It is also found in the placenta, in colourless corpuscles, and in embryonic tissues.

To obtain glycogen in considerable quantity, it is best to use the liver of a large rabbit. The animal must have been well fed on a

* Apparatus necessary: A solution of potassio-mercuric iodide, made by precipitating a solution of mercuric chloride with potassium iodide, washing the precipitate, and adding to it a boiling solution of potassium iodide till the latter is saturated. Any precipitate which occurs on cooling is to be filtered off. Dilute hydrochloric acid. Methylated spirit; ether; absolute alcohol. Large funnel and Swedish filter-papers. Large knife; capsule; several beakers; distilled water; ice. Mortar and pestle; large Bunsen's burner.

diet of grain and sugar for some days (preferably weeks) previously, and should have a full meal of grain, carrots, and sugar, about two hours before it is killed, in order that it may be in full digestion. The rabbit is killed either by decapitation or by a blow on the head, the abdomen is rapidly opened, and the liver is torn out and chopped up as quickly as possible with the knife, and is thrown, with the exception of a piece preserved to show the presence of sugar, into water which is kept boiling. This operation must be performed within half a minute of the death of the animal, and the water must not be allowed to fall below the boiling-point. The liver is to remain in the boiling water for five minutes; it is then placed in a mortar, reduced to a pulp, and again boiled in the capsule for ten minutes. The liquid is filtered, and the filtrate is rapidly cooled by placing the vessel in iced water. The albuminous substances in the cold filtrate are precipitated by adding potassio-mercuric iodide and dilute hydrochloric acid alternately as long as any precipitate is produced. The mixture is then stirred, is allowed to stand for five minutes, and is filtered. Alcohol is added to this second filtrate until glycogen is precipitated, which occurs after about 60 per cent. of absolute alcohol has been added. The precipitate is then filtered off and is washed with weak spirit, strong spirit, absolute alcohol (two or three times), and finally with ether. It is then dried on a glass plate at a moderate heat, and, if pure, should remain as a white amorphous powder. If the water has not been completely removed, the glycogen will form a gummy mass; in this case it must again be treated with absolute alcohol.

With some glycogen powder show that it reacts to the following **tests**:

It is freely soluble in water, the solution looking **opalescent** and giving a **port-wine** colouration with iodine, which disappears on heating and returns on cooling.

It is insoluble in absolute alcohol and in ether.

It is precipitated by basic lead acetate.

It is converted into sugar by ferments, or by boiling with dilute acids.

Glycogen, which is present in the fresh liver, very soon after death is changed into sugar. That this is the case may be shown by boiling the piece of liver which was put aside in the preparation of glycogen, and which was not at once thrown into boiling water; filtering, and testing the filtrate for sugar.

Dextrine:

Preparation.—Is made in commerce by heating dry potato-starch to a temperature of 200° C. It is also produced in the process of

the conversion of starch into sugar by diastase, and by the salivary and pancreatic ferments.

With dextrine in powder show the following **tests**:

It is a yellowish amorphous powder, white when pure, often with a peculiar smell.

It is freely soluble in hot or cold water, its solution is not opalescent.

It is insoluble in absolute alcohol and in ether.

It corresponds almost exactly in tests with glycogen; but one variety (achroo - dextrine) does not give the colouration with iodine.

Dextrose or grape-sugar occurs widely diffused in the vegetable kingdom, in fruits, honey, as well as in diabetic urine, in the blood, etc.; it is usually obtained from grape-juice, honey, or carrots.

It is crystalline, easily soluble in water; not so sweet as cane-sugar. It is dextro-rotatory.

It is not easily charred by strong sulphuric acid.

It is soluble in alcohol. It is precipitated by neutral or basic lead acetate in presence of amacosin.

With a solution of glucose show the following **tests**:

(i.) *Trommer's.*—Add an excess of caustic potash, and then a solution of copper sulphate, drop by drop, to the solution, in a test-tube, as long as the blue precipitate which forms re-dissolves on shaking the tube. Heat the upper portion of the fluid, and a yellowish-brown precipitate of copper suboxide appears.

The test may also be done with **Fehling's** solution (p. 240), or with acetic acid solution of neutral acetate of copper 2·5 per cent. (**Barford's** solution).

(ii.) *Moore's.*—Heat the solution of sugar in a test-tube with caustic potash; a brown colouration appears (? glucic and melassic acids).

(iii.) *Bismuth or Böttcher's test.*—Add a little bismuth oxide or subnitrate and an excess of caustic potash to the solution in a test-tube, and heat; the solution becomes at first gray and then black from reduction of the metal.

(iv.) *Picric acid test.*—To the solution add about a fourth of its bulk of picric acid (saturated solution) and an equal quantity of caustic potash, and boil; the liquid becomes of a very deep coffee-brown.

(v.) *Indigo carmine test.*—To the solution add a strong solution of sodium bicarbonate, and then a little indigo carmine. Warm: the blue colour disappears, the liquid

becoming violet and then yellow, but the original colour returns on shaking the test-tube.

(vi.) *Fermentation.*—If a solution of sugar be kept in a warm place for some time after the addition of yeast, the sugar is converted into alcohol and carbon dioxide :

$$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2.$$

(vii.) *Silver test.*—Add to a solution of dextrose some ammonio-nitrate of silver, and boil. The metallic silver is deposited as a mirror at the sides and bottom of test-tube.

(viii.) *Phenyl-hydrazin test.*—To a solution in a test-tube containing dextrose add a little solid sodium acetate, and a small amount of a solution of phenyl-hydrazin hydrochloride; place in boiling water for half an hour, and then cool under the tap; yellow crystals of phenyl-glucosazone will be thrown down as a precipitate.

(ix.) *Test with Saccharimeter.*—A solution of dextrose turns the plane of polarized light to the right.

Method of using the instrument.—Soleil's saccharimeter is employed for measuring the rotatory power of solutions of sugar. It consists of a strong glass tube nearly 4 inches (1 decimetre) in length, whose extremities are closed by glass caps, rendered water-tight by metallic screws. The tube is supported longitudinally between two Nicol's prisms, one of which acts as an analyzer, and the other as a polarizer. If the two prisms be turned so that their principal sections are at right angles to each other, and monochromatic light be looked at, the field will appear dark so long as the tube is not in position. As soon as the tube filled with syrup is interposed, the field becomes bright, and the analyzer has to be rotated through a certain number of degrees before the light disappears. The angle through which the analyzer has been rotated is measured on a scale attached to the instrument, and it is by this angle that the effect of the fluid upon the plane of polarized light is estimated. In actual practice, however, quartz prisms are interposed between the Nicol's, and the colour variations which result from their use are employed, as it is found to be difficult to decide the precise point at which the light disappears when the analyzer is being rotated.*

* Prof. M'Kendrick gives a full and lucid account of the Saccharimeter in his 'Text-Book of Physiology,' vol. i., pp. 66-70.

The polarimeter is almost exactly similar in its construction and use to the saccharimeter just described. It is employed for determining 'the specific rotatory power' of solutions of organic substances, such as proteids, which have the power of rotating the plane of the rays of polarized light.

(x.) *Naphthol or Thymol test.*—To a solution of dextrose a few drops of a 15 to 20 per cent. solution of α-naphthol, or of an alcoholic solution of thymol, should be added and thoroughly mixed by shaking in a test-tube; and a little of the mixed solution should then be poured into a small porcelain bowl, and rinsed round it. The bowl should then be allowed to drain, and afterwards should be dried carefully and again rinsed out with dilute solution of sulphuric acid. On drying a red colour appears, which is more violet if α-naphthol is used than thymol. The test is not characteristic of dextrose, as lactose and other sugars yield one more or less similar.

For the *Quantitative estimation of sugar*, see p. 240.

Lævulose is one of the products of the decomposition of cane-sugar, by means of dilute mineral acids, or by means of the ferment, *Invertin*, in the alimentary canal thus:

$$\underset{\text{Cane sugar}}{C_{12}H_{22}O_{11}} + \underset{\text{Water}}{H_2O} = \underset{\text{Dextrose}}{C_6H_{12}O_6} + \underset{\text{Lævulose}}{C_6H_{12}O_6}$$

Lævulose reacts to the same tests as dextrose; it is, however, non-crystallizable, and is lævo-rotatory. It is soluble in water and in alcohol. Its compound with lime is solid, whereas the dextrose-lime compound is not; thus the two sugars can be separated from one another when the two are made from cane-sugar.

Inosit is a non-fermentible variety of glucose occurring in the heart and voluntary muscles, as well as in beans and other plants.

It crystallizes in the form of large colourless monoclinic tables, which are soluble in water, but insoluble in alcohol or ether. It may be detected by evaporating the solution containing it nearly to dryness, and by then adding a small drop of a solution of mercuric nitrate, and afterwards evaporating carefully to dryness, a yellowish-white residue is obtained; on further cautiously heating, the yellow changes to a deep rose-colour, which disappears on cooling, but reappears on heating. If the inosit be almost pure, its solution may be evaporated nearly to dryness. After the addition of nitric acid, the residue mixed with a little ammonia and calcium chloride, and again evaporated, yields a rose-red colouration.

Lactose is contained in milk (p. 226).

It is less soluble in water than glucose. It crystallizes in rhombic prisms. It has a gritty and slightly sweet taste.

It is insoluble in absolute alcohol and in ether.

It undergoes alcoholic fermentation with extreme difficulty; it is first of all converted into galactose.

It gives tests similar to glucose, but less readily.

It requires 10 parts of a solution of lactose to reduce a quantity of Fehling's solution, which is reduced by 7 parts of a similar solution of glucose.

It is dextro-rotatory.

By means of a special micro-organism it is converted into lactic acid, and this may further be converted into butyric acid.

Maltose.—This sugar is particularly interesting from the fact that it is the variety chiefly formed in the digestion of starch by the salivary pancreatic juice.

It is crystallizable and soluble in water and in alcohol. Its tests are almost exactly similar to glucose; its reducing power, however, is one-third less; and it is said (when pure) not to reduce an acetic acid solution of copper acetate. It yields a crystalline phenyl-maltosazon, with phenyl-hydrazin, and may easily be converted into glucose by boiling with water, by dilute mineral acids, or by an inverting ferment.

Saccharose, or cane-sugar, is contained in the juices of many plants and fruits, and is usually extracted from the sugar-cane, from beet-root, or from the maple.

With an aqueous solution of *pure* cane-sugar show the following *tests:*

(i.) It crystallizes out from its solution on evaporation.
(ii.) It is precipitated on concentration by absolute alcohol.
(iii.) It forms a clear blue solution with potassium-hydrate and copper sulphate, but has no power to reduce the copper on boiling.
(iv.) It is dextro-rotatory.
(v.) It may easily be inverted by dilute mineral acids, or by the special ferment.
(vi.) It undergoes alcoholic fermentation only after inversion.
(vii.) It clears on the addition of sulphuric acid.
(viii.) It chars when heated with sodium or potassium-hydrate.

CHAPTER IV.

OILS AND FATS.

Oils and fats are neutral substances, with the composition of a compound ether, glycerine, $C_3H_5(OH)_3$ being the alcohol:

They are lighter than water; specific gravity ·91 to 94;

They give a greasy stain on paper;

They are insoluble in water; but are soluble in ether, chloroform, benzol, turpentine, or alcohol.

The chief fats of the body are:

Triolein, $C_3H_5(OC_{18}H_{33}O)_3$, is the most soluble of the fats, being soluble in both cold and hot alcohol or ether, and remaining fluid at 3° C.

Tristearin, $C_3H_5(OC_{18}H_{35}O)_3$, is the most insoluble, not dissolving in cold alcohol or ether, and not melting at a temperature below 53° C.

Tripalmitin, $C_3H_5(OC_{16}H_{31}O)_3$, is midway in solubility and melting point between the other two.

Glycerine.—A sweet-tasted viscid liquid, soluble in water and in alcohol; insoluble in ether.

Test.—Heat a little glycerine in a test-tube with some solid acid potassium sulphate; *acrolein*, a body of pungent smell, is produced ($C_3H_8O_3 - 2H_2O = C_3H_4O$).

Olive Oil, containing **triolein** or glycerine trioleate:

$$\begin{matrix} 3(C_{18}H_{33}O_2) \\ C_3H_5 \end{matrix}$$

(i.) Shake up a few drops of olive oil in a test-tube with water, and then allow it to stand; note that no emulsion or breaking up into very minute particles which do not run together takes place. Add ether, and shake the test-tube; note the solution of the oil in that reagent.

(ii.) To 10 cc. of a weak solution of potassium carbonate add a few drops of olive oil in a suitable test-tube, and shake

violently, note the formation of an emulsion; show also that an emulsion can be made by shaking up olive oil with a solution of egg-albumen in a test-tube.

(iii.) Add to some oil and water a few drops of caustic potash and warm. Complete solution takes place, a potassium oleate or soap is formed $C_3H_5(OC_{16}H_{31}O)_3+3KHO=C_3H_5(OH)_3+3C_{16}H_{21}O.OK$, and glycerine liberated. Both the soap and glycerine are soluble in water.

(iv.) Add some lead oxide to some olive oil and water in a porcelain basin, and apply heat, stirring the mixture. *Lead trioleate* is formed and glycerine is liberated (lead oleate is lead soap).

Fat (containing chiefly **tristearin** or glycerine tristearate):

(i.) Heat some fat, suet, lard or butter, in a test-tube, with water, until melted; add caustic soda. The fat is saponified, stearate (oleate and palmitate) of sodium and glycerine being formed.

(ii.) Add sodium chloride to the solution; *sodium stearate* separates out.

(iii.) Add sulphuric acid to another portion; sodium stearate is decomposed. *Stearic acid* separates out.

(iv.) To another portion add a solution of calcium chloride; *calcium stearate* is precipitated.

CHAPTER V.

THE URINE AND ITS CHIEF CONSTITUENTS.

Healthy Urine is a perfectly transparent amber-coloured liquid, with a peculiar and characteristic, but not disagreeable, odour, a bitterish taste, and a slightly acid reaction. The specific gravity varies from 1015 to 1025; under exceptional circumstances it may be as low as 1001, and as high as 1030. A light cloud of mucus from the urinary passages may often be seen in urine after standing.

Test its slightly acid reaction with litmus paper. Note that urine *does not alter the colour of a solution of Congo red*, and that it gives *no precipitate with sodium hypo-sulphite*, indicating that the acidity is not due to hippuric acid or to a free acid.

Note the changes which occur to the urine on standing for 1 or 2 hours and for 48 hours respectively. The acidity is first of all increased, but after a time it is replaced by alkalinity.

Take its specific gravity with the urinometer.

URINARY CONSTITUENTS.

The characteristic constituent of urine is

Urea (CON_2H_4).—Evaporate urine to half its bulk, filter, and add pure strong nitric acid; impure urea nitrate separates out. To obtain *pure* urea from this, purify it by re-crystallization with animal charcoal, and afterwards decompose it with barium carbonate into barium nitrate and urea. Evaporate the mixture to dryness and extract with hot alcohol. The pure urea will separate out from the alcoholic solution on cooling. Urea may also be obtained in the following ways:

Concentrate urine to a fourth of its bulk, and add a cold saturated solution of oxalic acid. This throws down crystals of oxalate; remove them and boil with some chalk and a little water. Oxalate of lime is precipitated, and can be filtered off together with the

excess of chalk. Carbonic acid is given off, and the urea remains in solution, and may be crystallized out by concentrating.

Or mix urine with animal charcoal and evaporate gently to dryness. Extract with alcohol and concentrate till a drop will crystallize, then set aside to cool.

Urea is freely soluble in water and in alcohol, but not in ether. Make a strong solution of urea in water, and use it for the following **tests :**

(i.) Allow a few drops to crystallize on a glass slide, and examine with the microscope. Note that the urea crystallizes out in transparent four-sided prismatic needles, terminated by one or two oblique facets.

(ii.) Either in a test-tube, or on a glass side, add a few drops of *pure* nitric acid to some of the urea solution, *Urea nitrate* separates out ($CON_2H_4.HNO_3$) in the form of six-sided tables. Examine the crystals with the microscope. To another portion of the urea solution add fuming nitric acid, effervescence occurs, due to the decomposition of urea thus :

$$CON_2H_4 + N_2O_3 = CO_2 + 2H_2O + 2N_4$$

(iii.) Proceed in a similar manner to ii., but use a concentrated solution of oxalic acid. *Urea oxalate* separates out ($CON_2H_4.C_2H_2O_4$) in the form of tabular plates, or prismatic bundles. Examine the crystals with the microscope.

iv.) To a concentrated solution of urea in a test-tube add mercuric nitrate solution ; a white precipitate of a compound of mercuric oxide and urea is formed.

(v.) Proceed as in iv., but previously add some common salt. The precipitate does not form until a large quantity of the re-agent has been added ; as the sodium chloride causes the mercuric nitrate to split up into mercuric chloride and sodium nitrate, and until the whole of the sodium chloride has been used up, there is no unchanged mercuric nitrate to produce the precipitate.

(vi.) Take some urea crystals in a test-tube and heat gently ; the urea melts and gives off ammonia ; heat further to dryness and to the residue ; add potassium hydrate solution and a small drop of copper sulphate ; a rose-red colour due to the presence of **biuret** appears :

$$\underset{\text{Urea}}{2CON_2H_4} - \underset{\text{Ammonia}}{NH_3} = \underset{\text{Biuret}}{C_2O_2N_3H_5}$$

Repeat the experiment, but apply heat for a longer time; cyanuric acid, which gives a violet colour with potassium hydrate and copper sulphate, remains:

$$\underset{\text{Biuret}}{3C_2O_2N_3H_5} - \underset{\text{Ammonia}}{3NH_3} = \underset{\text{Cyanuric acid}}{2C_3H_3N_3O_3}.$$

Uric Acid ($C_5H_4N_4O_3$) is another characteristic constituent of urine, from which it may be thus obtained: Add five or six drops of pure nitric or hydrochloric acid to two ounces of urine in a narrow urine glass, and allow it to stand for twenty-four hours. A brick-red coloured sediment of uric acid will be observed. Examine some under the microscope. Brown crystals of various sizes will be seen, the most characteristic being plates, rosettes, and lozenge-shaped.

Uric Acid is insoluble in cold water, very slightly soluble in hot, soluble in caustic alkaline solutions, forming urates; most easily soluble in solutions of lithium salts.

With uric acid powder show the following **tests**:

(i.) *Murexide test.*—Add a drop of strong nitric acid, evaporate to dryness over a water-bath, or at a temperature not exceeding 40° C. *Alloxan* ($C_4H_2N_2O_4$) is formed, as a reddish-coloured residue. Add a drop of ammonia solution with a glass rod, and the purple colour of *murexide* ($C_8H_8N_6O_6$) is produced. If potassium or sodium hydrate be used, the colour is more violet. The stages of the reaction are the following:

$$\underset{\text{Uric acid}}{2C_5H_4N_4O_3} + 2H_2O + O_2 = \underset{\text{Alloxan}}{2C_4H_2N_2O_4} + \underset{\text{Urea}}{2CON_2H_4};$$

By heat:

$$\underset{\text{Alloxan}}{3C_4H_2N_2O} = \underset{\text{Alloxantin}}{C_8H_4N_4O_7} + \underset{\text{Parabanic acid}}{C_3H_2N_2O_3} + CO_2$$

Murexide contains furfurate of ammonium ($C_8H_4(NH_4)N_5O_6$).

$$\underset{\text{Alloxantin}}{C_8H_4N_4O_2} + 2NH_3 = \underset{\text{Murexide}}{C_8H_8N_6O_6} + H_2$$

(ii.) *Schiff's test.*—Dissolve uric acid in a solution of sodium carbonate, and place a drop upon a filter-paper moistened with solution of silver nitrate; a black stain will result.

(For urates see p. 239.)

Hippuric Acid, in the form of hippurates ($C_9H_9NO_3$) is a constant constituent of human urine, but appears in greater amount in the urine of horses and other herbivora. It appears to arise from benzoic acid and other allied substances taken in as food. It is probably formed in the kidney.

Hippuric acid is only slightly soluble in water, soluble in alcohol and in ether; occurs in colourless, odourless, four-sided prismatic crystals, slightly bitter to the taste.

Take some crystals of hippuric acid for the following **tests**:

(1) Heat in a test-tube, oily red drops will form and benzoic acid sublime.

(2) Heat to dryness with nitric acid, a smell of nitro-benzine will be produced.

(3) Boil with Fehling's solution, some reduction of the copper will take place.

Kreatinine ($C_4H_7N_3O$) is also a constant constituent of human urine. It is derived from the kreatin of muscle. It is dehydrated kreatin, from which it may be obtained by boiling with water, or with hydrochloric acid.

Kreatinine crystalizes in large colourless prisms. It is soluble in water and in alcohol, but not in ether. It has an alkaline taste and reaction (?). It has the property of reducing copper solution, and forms a fine crystalline compound with zinc chloride. It is this property which is used as a test for kreatinine. The formula of this substance is $C_4H_7N_3OZnCl_2$, and is made up of fine needles.

To prepare kreatinine from urine: Add milk of lime and calcium chloride in excess to 250 cc. of urine. Remove the precipitated phosphates by filtration; evaporate the filtrate to the consistence of a syrup and add an equal amount of alcohol; put aside for twenty-four hours and filter; add 1 to 2 cc. of an alcoholic solution of zinc chloride. On standing for some time, the crystalline compound of kreatinine and zinc chloride will separate out, and may be removed and purified.

INORGANIC CONSTITUENTS.

The principal inorganic constituents of the urine are **phosphates**, in combination with calcium, magnesium, sodium and potassium; **chlorides**, almost entirely of sodium, but also of potassium; and **sulphates**, the chief being potassium sulphate.

Phosphates.—(1) Add to urine in a test-tube some potassium or sodium hydrate, and warm gently. A transparent flake precipitate will separate (earthy phosphates).

(2) Strongly acidulate with nitric acid, and then add some solution of ammonium molybdate and heat. A yellow precipitate will occur.

(3) Add to urine in a test-tube nitric acid, and then barium chloride; remove the precipitate of barium sulphate, then render alkaline, and remove the precipitate of baric phosphate.

(4) In the filtrate, after the removal of earthy phosphate by

filtration in (1), show the presence of alkaline phosphates not precipitated by alkalies, by test 2.

(5) Acidulate urine with acetic acid, and add a solution of an uranium salt. A bright yellow precipitate of ammonio-uranium phosphate will result.

Chlorides.—Acidulate the urine with strong nitric acid, and add a solution of silver nitrate. A precipitate of silver chloride, soluble in ammonium hydrate, will be thrown down.

Sulphates.—To urine acidulated with hydrochloric acid, add barium chloride solution. A precipitate of barium sulphate will occur, insoluble in nitric acid.

Colouring matter may be precipitated with the chlorides, sulphates, and phosphates on addition of lead acetate. The pigment may be extracted with alcohol, acidulated with sulphuric acid, and the extract is filtered off, and then is shaken up with chloroform, which takes up the pigment.

QUANTITATIVE ESTIMATION OF THE CHIEF CONSTITUENT.

Urea.—(i.) *Hypobromite method.*—One of the forms of apparatus* employed in this method (Russell and West's) consists of (*a*) a water-bath supported by three iron bands, arranged as a tripod. The bath is provided with a cylindrical depression, and with a hole, into which fits a perforated india-rubber cork; (*b*) a bulb tube with a constricted neck; (*c*) a glass rod provided with an india-rubber band at one extremity; (*d*) a pipette of five cubic centimetres capacity; (*e*) a graduated glass collecting tube; (*f*) a spirit lamp; (*g*) a wash-bottle with distilled water; (*h*) hypobromous solution. The hypobromous solution is made in the following way: three and a half ounces (100 grm.) of solid caustic soda is dissolved in nine ounces (250 grm.) of distilled water. When the solution is cold, seven drachms (25 cc.) of pure bromine are to be added carefully and gradually. The mixture is not to be filtered: it keeps badly, and for this reason it should be made shortly before it is required; or the aqueous sodium solution of hydrate may be made in large quantities as it does not undergo any change, the bromine in the proper proportion being added at the time it is required for use.

Method.—Fill the pipette to the mark on the stem with the urine to be examined; pour the 5 cc. of urine thus measured out into the bulb; fill up the bulb tube as far as the constricted neck with distilled water from the wash-bottle; insert the glass rod (*c*) in such a way that the india-rubber band at the extremity fills up

* Made by Cetti, Brook Street, W.C.

the constricted neck; the diluted urine should exactly occupy the bulb and neck of the tube, no bubble of air being below the elastic band on the one hand, whilst on the other the fluid should not rise above the band; in the former case a little more water should be added, in the latter a fresh portion of urine must be used, and the experiment repeated. After adjusting the glass rod, fill up the rest of the bulb tube with hypobromous solution; it will not mix with the urine so long as the rod is in place. The water-bath having been previously erected, and the india-rubber cork fixed firmly into the aperture, the bulb tube is to be thrust from below through the perforation in the cork. The greater part of the tube is then beneath the water-bath, the upper extremity alone being grasped by the cork. Half fill the water-bath with water, fill also the graduated glass tube (*e*) with water, and invert it in the bath; in doing this no air must enter the tube, which, when inverted, should be completely filled with water. Now slide the graduated tube towards the orifice of the bulb tube, at the same time withdrawing the glass rod which projects into the bath through the cork. At the instant that the rod is withdrawn, the hypobromous solution mixes with the diluted urine, and a decomposition takes place represented thus:

$$\underset{\text{Urea}}{CON_2H_4} + \underset{\substack{\text{Sodium}\\\text{hypobromite}}}{3NaBrO} + \underset{\substack{\text{Caustic}\\\text{soda}}}{2NaHO} =$$

$$\underset{\substack{\text{Sodium}\\\text{bromide}}}{3NaBr} + \underset{\text{Water}}{3H_2O} + \underset{\substack{\text{Sodium}\\\text{carbonate}}}{Na_2CO_3} + \underset{\text{Nitrogen}}{N_2}$$

The nitrogen produced is given off as gas, and displaces the water in the graduated tube, which is held over it. The gas is at first evolved briskly, but afterwards more slowly; to facilitate its evolution, the bulb of the tube may be *slightly warmed* with a spirit-lamp; as a rule, however, this is unnecessary. After ten minutes, the amount of water displaced by the gas should be read off on the tube, which is divided into tenths. Each number on the tube represents one grm. of urea in 100 cc. of urine. Normal urine should yield roughly 1·5-2·5 parts of nitrogen by this test. If 5 cc. of urine gives off more nitrogen than fills the tube to iii., the urine should be diluted with an equal volume of water, and take 5 cc.; read off and multiply by two. If the urine contain albumen, heat it with two or three drops of acetic acid, filter, and take 5 cc. of the filtrate. In the ordinary way, when the urea is estimated by decomposition method, allowance must be made for variations of the temperature and pressure. In Russell and West's apparatus, with

the use of a 2 per cent. solution of hypobromite, there is a deficit of about 8 per cent., as 1 grm. of urea gives up only 343 vols., instead of 373 vols., of N ; but this deficit is practically covered by making no correction for the temperature and pressure.* The formula for the correction, which has to be used under other circumstances, being (according to Foster and Langley) the following: v′=vol. in cubic centimetres of the N obtained, at temperature $t°$ C and pressure B in mm. of mercury, and T the tension of aqueous vapour at $t°$ C the volume V at O° C, and 760 mm. pressure:

$$= 1+\frac{V'}{\cdot 003665\ t} \times \frac{B-T}{760}$$

·003665 being the co-efficient of gases.

(ii.) *Liebig's Method.*—This method is of greater accuracy. *The solutions required are :*

(*a*) *Baryta mixture*=2 vols. of saturated solution of barium nitrate and 1 vol. of saturated solution of barium hydrate ;

(*b*) A standard solution of *mercuric nitrate*, such that 1 cc. will precipitate 0·01 grm. of urea ; and

(*c*) A solution of *carbonate of soda.*

Method.—Take 40 cc. of urine, add 20 cc. of (*a*), filter off the precipitate of sulphates and phosphates ; keep the filtrate. Fill a burette with (*b*), and take 15 cc. of the filtrate in a dish. Let (*b*) fall drop by drop into the 15 cc. in the dish, stirring constantly. Have ready a glass plate with several separate drops of (*c*), and from time to time add a drop of the urine mixture by means of a glass rod to one of the drops. When a yellow colour *first* appears in a drop of the sodium carbonate, the mercuric nitrate is just in excess. Read the burette. Calculate as follows :

1 cc. of mercuric solution precipitates 0·01 grm. of urea ∴ the No. of cc. used × 0·01 = amount of urea in 15 cc. of filtrate—*i.e.*, in 10 cc. of urine. But 10 cc. of urine usually contains enough chloride of soda to act on 2 cc. of mercuric nitrate solution. Hence, *when reckoning the number of cc. of standard mercury solution used, a deduction of* 2 *cc. must always be made.*

The most accurate application of this method, at any rate when the percentage of urea is about 2 per cent., is, first of all, to estimate the amount of chlorides present, and then to reduce the amount of urea 1 grm. for every 1·3 grms. of chloride present. If the urea is below 2 per cent., 0·1 cc. of mercuric nitrate must be deducted for every 4 cc. of the solution used.

ESTIMATION OF URIC ACID.

For the following account we are indebted to our friend and colleague, Dr. A. Haig.

Estimation of Uric Acid.—'The most useful process clinically is probably that of Haycraft, originally published in the *British Medical Journal* (vol. ii., 1885, p. 1100. See also same *Journal*, vol. ii., 1891, p. 10, and *Zeitschr. für physiolog. Chem.*, May, 1891).

'By this process the uric acid is combined with silver, forming a gelatinous precipitate, which is separated by filtration and washing. It is then got into solution in nitric acid, and the silver is estimated by a colour test (method of Volhard); the uric acid is calculated from the silver found. All solutions can be obtained from Messrs. Savory and Moore, New Bond Street, W.

'For the filtration process an air-pump driven by water or mercury is required; without this help filtration of the gelatinous urate of silver would take hours; with it the whole process can be completed in about half an hour.

'I have used this process almost continuously since its publication, and must have repeated it between 2,000 and 3,000 times. I believe it may compare as regards accuracy with any process that is known, and that, with a relatively small experience, results may be obtained which are strictly comparable.

'I have applied this process not only to urine, but also to the estimation of urate in watery extracts of blood organs and tissues, these being obtained by a method given in Solkowski and Leube (*Die Lehre vom Harn*, p. 94. See also *British Medical Journal*, January, 1891).

'The chief points to be attended to are: (1) To get all deposits in solution by the aid of dilution, warmth, and alkali before taking a sample. (2) To add bicarbonate of soda and the nitrate of silver solution to the full extent directed. (3) Not to grudge time to the filtering and washing process. (4) Not to dilute more than is necessary, as dilution causes loss of urate in the filtration process.'

Quantitative Estimation of Chlorides:

(i.) *Liebig's Method.—The solutions required are:*

(*a*) *Baryta mixture* as above; and

(*b*) A standard solution of *mercuric nitrate*, such that 1 cc. would be capable of decomposing 0·01 grm. of sodium chloride.

Method.—Take 40 cc. of urine free from albumen, and add 20 cc. of (*a*). Filter. Take 15 cc. of the filtrate and place it in a flask or dish, adding a drop or two of nitric acid. Fill a burette with (*b*), and slowly run some of this solution into the filtrate in the dish, stirring constantly. *As soon as* a distinct cloud appears in the diluted urine, and does not disappear on stirring, then all the sodium chloride in the urine has been decomposed. Read the burette. Calculate as follows :

1 cc. of mercury solution decomposed 0·01 grm. of sodium chloride ∴ the number of cc. used × 0·01 grm. = number of grms. of sodium chloride in 15 cc. of filtrate—*i.e.*, 10 cc. of urine.

(ii.) *Silver Method.—The solutions required are :*

(*a*) Solution of *silver nitrate*, containing 29·075 grms. of the fused salt in 1000 cc. of distilled water; of this solution 1 cc.=0·01 grms. of sodium chloride.

(*b*) Saturated solution of *potassium chromate* (neutral).

Method.—Take 10 cc. of the urine, and dilute with 100 cc. of distilled water. Add to it a few drops of solution (*b*). To this mixture in a beaker allow the standard solution (*a*) to drop in from a burette. A precipitate of silver chloride will occur as long as any chloride is uncombined. When the whole of the chloride is satisfied, a reddish or pink (since there is much white precipitate present) precipitate of silver chromate appears. This indicates the time to stop the addition of the silver nitrate, and the amount of the solution which has been used is read off. This will indicate the amount of silver nitrate necessary to convert all the chlorine present in 10 cc. into silver chloride. It is known that 1 cc. of the solution = 0·005837 grm. of salt, and from this the total amount of chloride present can be estimated. A correction should be made by subtracting 1 cc. of the silver solution used, as the white contain certain other substances more easily precipitated than the chromate.

Quantitative Estimation of Phosphates:

The solutions required are :

(*a*) Solution of *sodium acetate*, containing 100 grm. of sodium acetate, 100 cc. of acetic acid, and 900 cc. of distilled water;

(*b*) A solution of *uranium acetate* or *nitrate*, such that 1 cc. will precipitate 0·005 grm. of phosphoric acid; and

(*c*) A solution of *ferrocyanide of potassium.*

Method.—Take 50 cc. of urine. Add some (*a*) solution, and heat on a water-bath to nearly 100° C. Fill the burette with (*b*),

and add this slowly to the urine. Have ready a glass plate with several distinct drops of potassium ferrocyanide solution. From time to time add a drop of the urine mixture to one of these drops; and when there FIRST appears a reddish-brown colour in a drop of potassium ferrocyanide, all the phosphates are precipitated. Read the burette. Calculate thus:

1 cc. precipitates 0·005 grm. of phosphoric acid ∴ the number of cc. used × 0·005 grm. = number of grms. of phosphoric acid *in* 50 *cc. of urine.*

CHAPTER V.

THE BLOOD.

(i.) Test the alkaline **reaction** of the blood in the following way, as recommended by Schäfer:—A drop of blood, obtained by pricking the finger, is placed upon the smooth-coloured surface of a piece of dry, faintly reddened, glazed litmus-paper, and after a few seconds is wiped off with the corner of a handkerchief or clean linen rag moistened with water. The place where the blood has stood is seen to be marked out as a well-defined blue patch upon the red or violet ground.

(ii.) Show that the **coagulation is retarded** by freezing and by the presence of neutral salts. Draw a few drops of blood from the finger into a watch-glass previously cooled in a freezing mixture; no coagulation takes place at the freezing temperature. Add a drop of blood to a little saturated solution of sulphate of soda in a watch-glass; the blood does not coagulate.

Two cc. of blood are placed in a platinum capsule, which is surrounded by alternate layers of pounded ice and salt. The capsule is allowed to remain until the blood is frozen; it is then removed, and the solid mass of frozen blood is gradually thawed. The blood on again becoming liquid will be found to be darker in colour and more transparent than it was previous to congelation. It is in the *lake* condition, owing to the discharge of the hæmoglobin from the corpuscles into the plasma.

The **tests** for blood are (iii.) the examination by the spectroscope, when oxyhæmoglobin and its derivatives give characteristic absorption bands, to be presently mentioned (p. 218).

(iv.) The formation of oxyhæmoglobin crystals.

(v.) The formation of hæmin crystals (p. 83).

(vi.) *The guaiac test.*—A drop or two of tincture of guaiacum is shaken up with the suspected fluid; to the mixture some ozonic ether is added; if blood be present, a sapphire-blue colour appears

at the junction of the fluids, and gradually mounts upwards through the ozonic ether, bubbles of gas being at the same time evolved.

HÆMOGLOBIN AND ITS DERIVATIVES.

The Spectroscope is an instrument which is largely used in physiological research in connection with the pigments of the body. It consists of a table, upon which is placed a prism. Two tubes are attached to the edge of the table—one, the collimator, whose extremity is directed towards the solution whose pigment is to be examined; the other, a telescope with which to examine the resulting spectrum. Both tubes are movable round the edge of the

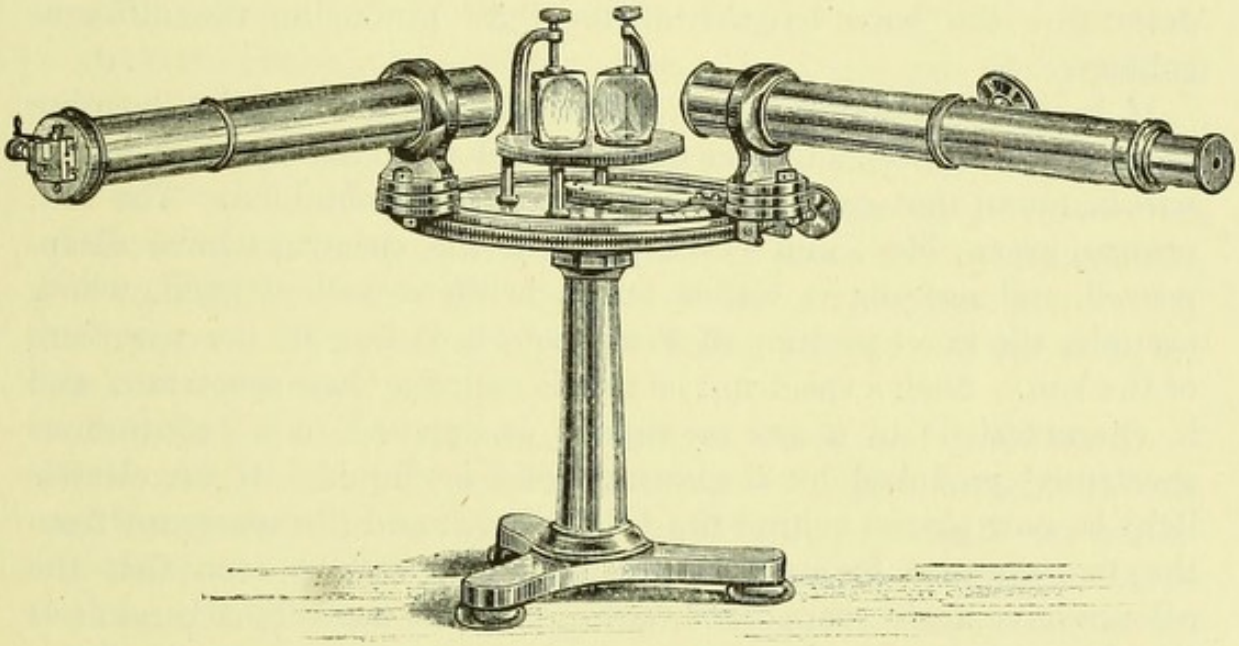

Fig. 68.—Spectroscope.

table, and each can be clamped in any desired position. The collimator is provided with a slit, whose width can be adjusted so as to give a greater or less amount of light; it also has a lens, whose focus can be altered at will, and by its means all rays of light passing through it to the lens are rendered parallel before they enter the prism. The telescope is focussed in the ordinary way by means of a rack and pinion. When the spectrum is being examined a black cloth is thrown over the prism and the ends of the tubes so as to shut out all extraneous light. A ray of sunlight is then reflected into the distal end of the collimator, and the eye is applied to the ocular of the telescope.

The slit is increased or diminished in size and the telescope is adjusted by means of the screw until a sharply-defined spectrum or

band of colours is obtained. At first sight this band appears to be continuous like the rainbow, but upon closer examination it is seen to be traversed at right angles to its long axis by various dark lines, the distinctness of which is proportional to the strength of the spectroscope employed and to the accuracy with which it is focussed. These lines, which are called the 'absorption bands,' were originally classified by Fraunhofer, their discoverer, as the A B C lines, situated in the red part of the spectrum; the D, which is really a double line, on the orange side of the yellow; E in the green; F in the blue; G at the beginning; and H^1 and H^2 near the end of the violet. As these lines occupy fixed positions they were used as indices for spectral comparisons; but physicists now employ a more accurate means of registration, since it has been found possible to determine the wave-lengths of the light producing the different colours.

If in place of daylight the yellow light produced by burning sodium in a Bunsen's burner be examined with the spectroscope, it will be found that an entirely different result is obtained. The red, orange, green, blue, and violet parts of the spectrum have disappeared, and nothing is visible but a brilliant yellow band, which occupies the exact position of Fraunhofer's D line in the spectrum of the sun. Such a spectrum as this is called a 'line spectrum,' and is characteristic of a gas or vapour as opposed to a 'continuous spectrum' produced by a glowing solid or liquid. If an electric light be now placed behind the yellow light, and the spectrum from the two sources be examined together, it will be seen that the colours have again reappeared, but in place of the bright band at D there is a dark 'absorption band,' which is of exactly the same character as that seen in the sun's spectrum. Such a spectrum as this is, therefore, called an 'absorption spectrum,' and this variety is the only one met with in physiological research.

ABSORPTION SPECTRA OF HÆMOGLOBIN AND ITS DERIVATIVES.

(i.) Dilute defibrinated blood with ten or twelve times its bulk of distilled water, place some of the solution in a test-tube (12 cm. × 2 cm.) or other suitable vessel, and examine with spectroscope. The whole of the spectrum will be seen to be cut off except the red. Dilute some of the above dilution four times, and it will be found that an absorption band extends from D (sodium band) to a little beyond E (middle of green); but that the red, orange, yellow, and part of the green will be seen. On further dilution (five times and beyond) the broad band will be found to be replaced by two narrower bands separated by a green interspace. Of the two bands, the one

to the left, next to D, is the narrower and deeper. On extreme dilution with water both of the bands disappear, but of the two the band D persists the longer. Before the bands disappear they are seen as faint shadows. These appearances indicate the presence of **oxyhæmoglobin.**

(ii.) To a solution of blood (1 in 20) add a few drops of ammonium sulphide,* and thoroughly mix; the solution becomes purplish, and on spectroscopic examination the single band of **reduced hæmoglobin** will be seen. It is fainter and with less sharply defined edges than those of oxyhæmoglobin, and it extends from D to E, none of the green being visible in the midst. On dilution the band soon disappears, but as long as it remains it continues single. There is in addition more absorption to the left and less to the right end than in (i.).

(iii.) Pass carbon monoxide for some time through blood diluted as in (ii.); as coal-gas contains a large proportion of carbon monoxide, that gas may be used if more convenient. On examination the two bands of **carbonic oxide hæmoglobin** may be observed. These bands are almost exactly similar to those of oxyhæmoglobin, but are slightly nearer the violet end, and are besides more equal and deeper. The solution resists reducing agents. Nitric oxide gas has much the same effect.

(iv.) To a further portion of diluted blood add some drops of acetic acid, and boil for some minutes. The liquid becomes brown, and the spectrum shows a distinct band in the middle of the red (to the right of C), indicating the presence of **acid hæmatin.** After cooling, add ether and shake up well. The ether on settling at the top is seen to be of a brown colour, and on examination shows three bands, besides the C band—viz., a very faint narrow band to right of D, a broad band to left of E, also a faint and third about midway between E and F.

(v.) As in (iv.), but add excess of ammonia; a band is seen in the red, but nearer D (**alkaline hæmatin**).

(vi.) To liquid (v.) add a reducing agent; a spectrum showing two bands somewhat like those of oxyhæmoglobin, but more to the right, is seen: **Stokes' reduced hæmatin or hæmo-chromagen.**

(vii.) Dissolve some dried blood in saline solution. It will show a spectrum of **methæmoglobin**, consisting of the two bands of oxyhæmoglobin; another band in the red near C, like that of (iv.) On

* This we find to be the most convenient reducing agent, but Stokes' fluid, a solution of ferrous sulphate, to which is added tartaric acid, and then ammonium hydrate, until the reaction is alkaline, or a solution of a stannous salt, treated in the same way, may be used.

the addition of ammonia, the last band is replaced by one close to the left of D. On addition of ammonium sulphide, the spectrum of reduced hæmoglobin will appear, and on shaking with air that of oxyhæmoglobin.

(viii.) Dissolve in a test-tube fresh defibrinated blood drop by drop to strong sulphuric acid, and, if necessary, filter through asbestos; a beautiful deep red-coloured fluid is the result—*hæmatoporphyrin*. It gives two bands—one at D, and a dark, well-defined band about midway between D and E.

Estimation of the Amount of Hæmoglobin.—A method, differing from that described (p. 80), of approximately

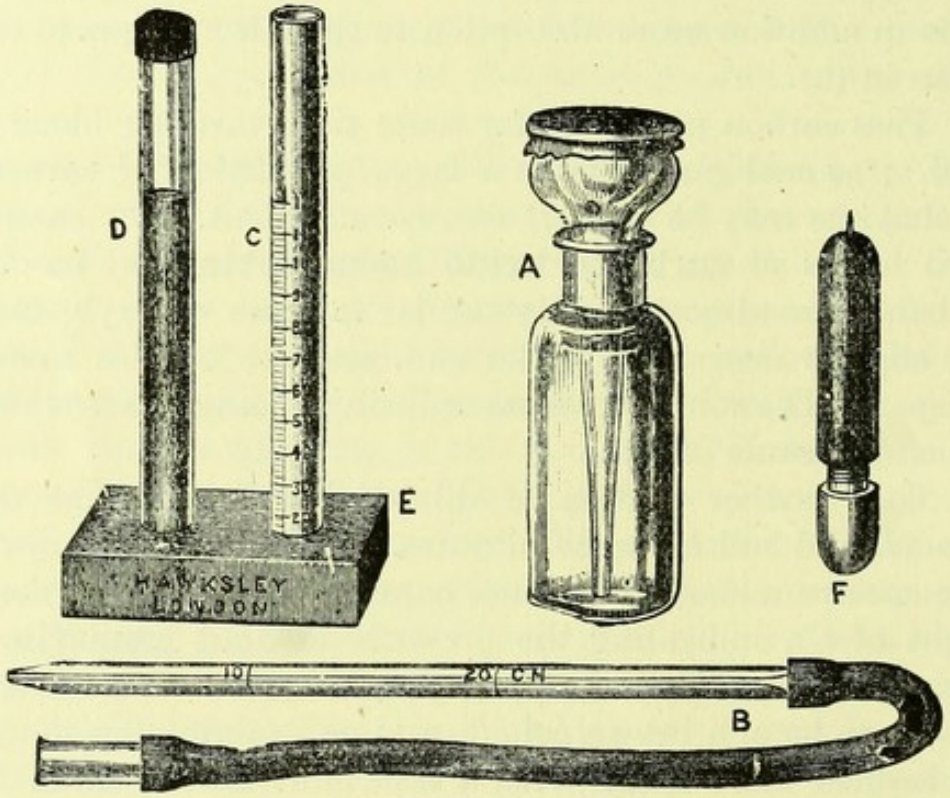

Fig. 69.—Gowers' Hæmoglobinometer.

estimating the amount of the hæmoglobin in blood has been devised by Gowers with an instrument called a *hæmoglobinometer*.* The theory of the apparatus consists in comparing a given sample of the blood to be examined, diluted with given quantities of water, with a standard colour solution representing the colour of a normal (1 per cent.) solution of blood. The apparatus consists of two glass tubes of exactly the same size. One contains (D) a standard of the tint of a dilution of 20 cmm. of blood in 2 cc. of water (1 in 100), composed of glycerine jelly tinted with carmine and picrocarmin. The second tube (C) is graduated, 100 degrees being equal to 2 c. (100 times 20 cmm.).

* Made by Hawkesley, Oxford Street.

The 20 cmm. of blood are measured by a capillary pipette (B) (similar to, but larger than, that used for the hæmacytometer). This quantity of the blood to be tested is ejected into the bottom of the tube, into which a few drops of distilled water have been previously placed. The mixture is rapidly agitated to prevent the coagulation of the blood. Distilled water is then added drop by drop (from the pipette stopper of a bottle (A) supplied for that purpose), until the tint of the dilution is the same as that of the standard, and the amount of water which has been added (*i.e.*, the degrees of dilution) indicates the amount of hæmoglobin.

Since average normal blood yields the tint of the standard at 100 degrees of dilution, the number of degrees of dilution necessary to obtain the same tint with a given specimen of blood is the percentage proportion of the hæmoglobin contained in it, compared with the normal. By ascertaining with the hæmacytometer the corpuscular richness of the blood, we are able to compare the two. A fraction, of which the numerator is the percentage of hæmoglobin, and the denominator the percentage of corpuscles, gives at once the average value per corpuscle. In using the instrument, the tint may be estimated by placing a piece of white paper behind the tubes; some light is, however, reflected from the suspended corpuscles from which the hæmoglobin has been dissolved. It will be found that during six or eight degrees of dilution it is difficult to distinguish a difference between the tint of the tubes. It is therefore necessary to note the degree at which the colour of the dilution ceases to be deeper than the standard, and also that at which it is distinctly paler. The degree midway between these two will represent the hæmoglobin percentage.

BLOOD PLASMA. (Liquor Sanguinis.)

(1) Obtain plasma in one of the following ways:*

(α) Draw horse's blood into a narrow glass vessel which has been previously cooled by immersion in ice-cold water, and keep it at a temperature of from 0° to 2° C. for twenty-four hours. In that time the corpuscles will have subsided, and the supernatant fluid, or **pure plasma**, may be removed by careful decantation.

(β) Draw blood, preferably from a horse, into an equal quantity of a saturated solution of sodium sulphate (or of a 10 per cent. solution of sodium chloride, or into a fourth of its bulk of a saturated solution of magnesium sulphate), and

* It will be found that the second method is the easiest.

mix gently with a glass rod. Place the solution in a cool place for twenty-four to thirty-six hours, and allow the corpuscles to settle. The supernatant fluid is so-called **salted plasma.**

(γ) Inject a solution of commercial peptone into the jugular or other vein of a dog (in the proportion of 0·3 gramme of peptone to each kilogramme of body-weight), and then kill the animal by bleeding. The blood will be found to have lost its power of spontaneous coagulability; and on keeping in a suitable vessel in a cool place, or better on subjecting it to a centrifugal machine, will separate into an upper stratum of so-called **peptone plasma**, and a lower stratum of corpuscles.

(2) Take some of (α) plasma, and expose it to the ordinary temperature of the air, and in the course of a few minutes it will clot.

(3) Take 10 cc. of (β) plasma, and add to it 30-40 cc. of distilled water in a large test-tube; place in a warm chamber at 40° C. In a variable time, from minutes to hours, coagulation will ensue.

(4) Take some peptone-plasma, and pass through it a stream of carbonic acid gas; it will coagulate. (It should be noted that if peptone-plasma be subjected for some time to a temperature of 0° C., a proteid deposit of rounded granules not unlike blood-plates will be deposited.)

(5) Saturate plasma (either pure or salted) with sodium chloride or magnesium sulphate, and shake violently. A precipitate of **plasmine** will fall; separate by decantation after standing and filtration.

(6) Dissolve some of the plasmine scraped from the filter-paper in dilute saline solution, which may be made by adding to it some distilled water, the adhering salt being sufficient to form a saline solution with it. Set aside the solution for some time in a warm place; it will clot.

(7) Add to 10 cc. of plasma 10 cc. of a saturated solution of sodium chloride. A precipitate of **fibrinogen** will occur; remove the precipitate by filtration, and dilute the filtrate with two or three times its bulk of distilled water; it will not clot.

(8) Take the precipitate of fibrinogen, and dissolve it in dilute saline solution, as in (6), and show that it coagulates at a temperature of 56° C.

(9) Show that the solution of fibrinogen, on the addition of an aqueous solution of fibrin ferment, will, in the presence of a trace of calcium sulphate, clot.

Fibrin ferment may be obtained in the following manner:

Serum, or plasma, is mixed with over ten times its bulk of absolute alcohol, and the proteids which are precipitated are kept for several (six to eight) weeks in the alcohol. At the end of that time the precipitate is removed by filtration, washed with absolute alcohol, dried over sulphuric acid, and powdered. The powder is extracted with water, and by this means an aqueous solution of fibrin ferment is obtained. This aqueous solution will cause coagulation in such fluids as pericardial exudations, hydrocele fluid, or diluted plasma, not spontaneously coagulable, but containing fibrinogen.

SERUM.

Serum may be obtained by allowing pure plasma, or, better, fresh blood, to clot. The blood of almost any mammal may be used, particularly the horse, the ox, the pig, or the sheep. The blood is received into a tall glass vessel, previously rendered aseptic, until the vessel is quite full; the latter is then closed by a closely-fitting and greased stopper. The vessel should be set aside in a cool place for twenty-four to forty-eight hours, at the end of which time, on the removal of the stopper, serum, free from blood corpuscles, and almost free, too, from hæmoglobin, may be obtained by careful decantation, or by use of a syphon.

Serum will be found to be an almost clear, pale to dark, straw-coloured alkaline fluid, with a saltish taste, and a more or less characteristic smell; its specific gravity is about 1028.

(10) Take some clear serum in a large test-tube, and add to it an excess of solid magnesium sulphate or sodium chloride. Shake violently. A flocculent precipitate of **paraglobulin** is obtained; separate it by filtration.

(11) Dissolve the precipitate by washing the filter containing it with a small amount of distilled water as above (6), and with part of the solution ascertain the temperature at which the globulin coagulates. Retain the remainder (α).

(12) In the filtrate from (10) show the presence of **serum-albumin** by heat, noting the temperature at which it coagulates. Filter.

(13) Test the filtrate from (12) for any **proteid remainder**, also for **chlorides** and **phosphates**.

(14) Dilute serum with ten times its volume of water and pass a brisk stream of carbon dioxide gas through the solution; a precipitate of paraglobulin will appear. Separate by filtration, and dissolve as in (11). To one portion of the solution apply the globulin tests; retain the second portion (β).

HYDROCELE FLUID.

(15) Take some hydrocele fluid, and treat as in (10); a precipitate of **fibrinogen** occurs. Separate it.

(16) Dissolve fibrinogen, as in (11), and proceed in exactly similar manner; and taking one portion of solution, find the temperature at which it coagulates, and retain the other portion (γ).

(17) As in (12), show the presence of serum-albumen, and after its precipitation and removal by heat and filtration, test the filtrate for any proteid residuum, and for chlorides.

(18) Precipitate fibrinogen by the carbonic acid method, as in (14); apply the globulin tests to one portion, and retain the other (δ).

COAGULATION.

(19) Take fresh serum, and add it to an equal quantity of fresh, clear hydrocele fluid (faint yellowish to green in colour, S.G. 1016-1022), and keep it at a temperature of about 37° C. After a variable time it will clot.

(20) Add together solutions (α) and (γ); clotting will most likely occur (if the fluids from which they have been obtained were fresh).

(21) Add together solutions (β) and (δ); clotting does not occur.

(22) Treat plasma with an equal volume of 13 per cent. solution of sodium chloride; fibrinogen will be separated; remove by filtration, and dissolve as above, and then add solid sodium chloride to saturation, and paraglobulin falls. Remove by filtration, and dissolve. The solutions when mixed should after a time clot.

FIBRIN.

Fibrin should be obtained from blood or from pure plasma by whipping with a handle of twigs; the adhering filaments in the former case should then be thoroughly washed in a large quantity of water until quite colourless.

It is a soft, white, fibrous, and very elastic substance, and differs from all other proteids, in having a filamentous structure. Examine a piece of fibrin with the microscope.

It is insoluble in water and dilute saline solutions, slightly soluble in concentrated saline solutions, soluble on boiling in strong acids and alkalies.

(For other properties of fibrin see pp. 191, 192.)

MILK.

Milk is an opaque white fluid:

(i.) Examine a drop of cow's milk under the microscope with a high power. See that it consists of fat globules of different sizes in a clear fluid, *i.e.*, that it is an **emulsion**. Add dilute acetic acid by irrigation, and observe the coalescence of the globules owing to the excess of acid dissolving their caseinogen membrane.

(ii.) Test the alkaline **reaction** of *fresh* milk (London milk may be slightly acid); it becomes acid on standing, owing to the formation of lactic acid by fermentation, whilst in consequence the caseinogen separates.

(iii.) Take the **specific gravity** of fresh milk with the hydrometer, 1025-30—skimmed milk is higher. To 50 cc. of skimmed milk add water gradually from a burette till the specific gravity is brought down to normal. Read off the amount of water required.

The *constituents* whose presence is to be demonstrated are *oil* or *fat*, *caseinogen*, *lact-albumin*, *lactose* or *milk sugar*, and *salts*.

(iv.) To a portion of milk, add its own volume of caustic potash or soda, and warm the solution *gently;* the oil globules will be set free from their enveloping casein. Cool, add ether, shake the mixture, and allow it to stand. The **fat** will be dissolved in the ether, and will form with it a clear superstratum. Remove the transparent top layer with a pipette, evaporate off the ether; the oil will be left, and will give the *characteristic greasy spot when dropped upon paper*. The fats of milk are in addition to triolein, tristearin and tripalmitin, small quantities of the triglycerides of butyric, caproic, caprylic, and other acids; also lecithin, cholesterin, and a yellow lipochrome.

(v.) Dilute some of the milk with its own bulk of water; add a few drops of dilute acetic acid until a slight granular precipitate is formed. Warm the solution *gently* to 40° C., and a copious flocculent precipitate of **caseinogen** with adhering fat will appear. Filter off, and label the precipitate 'A.' Saturate another portion of diluted milk with magnesium sulphate or sodium chloride; a precipitate of caseinogen with adhering fat will occur.

(vi.) Boil the clear filtrate (from A); a coagulum of **lact-**

albumin will be formed. Filter this off, and label the precipitate 'B.'

Exactly neutralize the clear filtrate (from B) with caustic potash or soda; a precipitate of **acid-albumin**, which is soluble in acids, may appear.* Filter off the precipitate, and label it 'C.'

(vii.) *Lactose.*—Test the clear filtrate (from C) by Trommer's test for sugar (p. 203).

(viii.) Wash the precipitate A with dilute acetic acid, and afterwards with distilled water. Dissolve it in lime-water and an opalescent solution is obtained. Add strong nitric acid to one portion of this solution, and boil, a yellow precipitate falls. After cooling, add strong ammonia, the colour deepens to orange. This is the *xanthoproteic reaction;* it shows that a proteid is present.

(ix.) To another portion of the opalescent solution add rennet, no coagulation occurs. Pour in a few drops of 0·5 per cent. phosphoric acid, and then a little more rennet, a firm curd is produced, which subsequently contracts and squeeze out a clear whey. Rennet, when calcium phosphate is present, has the power of converting caseinogen into casein. This experiment shows that the proteid demonstrated by (viii.) is *caseinogen.*

(x.) Test the precipitate on filters B and C by boiling with Millon's reagent, a pink colouration will in each case be produced, showing that albumen is present.

In the case of B this albumen is either serum-albumin, or lact-albumin. It can be shown by a process of fractional heat coagulation to be lact-albumin.†

(xi.) Test a second portion of the clear filtrate from C for (i.) *phosphates*, by the addition of ammonio-sulphate of magnesia; a precipitate is formed: also by the molybdate of ammonium test; and for (ii.) *chlorides* by the addition of silver nitrate to another portion which has been acidulated with nitric acid; a curdy precipitate will fall, soluble in ammonia.

(xii.) To diluted milk add gastric or pancreatic rennet solution; the milk is curdled, or, in other words, casein separates

* This acid albumin has been produced by the previous acidification of the milk in the precipitation of casein.

† This process, though simple, is not adapted for a class. The senior student will find the details in a very interesting paper by Prof. Halliburton, published in the *Journal of Physiology*, vol. xi., 452, from which most of the facts given in the text are derived.

out. Collect the whey; then saturate it with sulphate of magnesium. A precipitate of **whey proteid** is produced. Wash the precipitate with a saturated solution of magnesium sulphate and dissolve it in water. Boil the aqueous solution, it becomes opalescent, but there is no precipitate; allow it to cool, and the opalescence disappears, therefore it is not a globulin. Add acetic acid, it is not precipitated; add rennet, there is no clot, therefore it cannot be caseinogen. Saturate its solution with ammonium sulphate, it is precipitated, and therefore it is not a peptone. With caustic potash and a trace of copper sulphate, it gives a violet, and not a pink reaction. Halliburton believes that this body is allied to the globulins, though it does not actually belong to this group of proteids.

CHAPTER VII.

THE BILE.

The bile is a more or less viscid fluid of a colour varying from deep yellow to green or brown.

(i.) Test its **reaction**. It is alkaline or neutral; and its **specific gravity** 1018-20.

(ii.) Neutralize and boil. There is no precipitate, which indicates the absence of native-albumins and globulins.

(iii.) Dilute with water and acidify with acetic acid. There will be a precipitate of **mucin** * or **nucleo-albumin**. Filter. (Dissolve the precipitate on the filter in lime-water, and test with *Millon's reagent* and with *lead acetate*).

(iv.) To a portion of the filtrate from (iii.) apply Gmelin's test for **bile pigments**. Place a drop of bile on a white plate, and add a drop of strong yellow nitric acid to it. A play of colours is produced. Green, blue, violet, red, and yellow appear in succession.

(v.) To another portion of the filtrate apply Pettenkofer's test for **bile salts**. These salts are glycocholate and taurocholate of sodium, and consist of sodium compounds of cholalic acid, glycin (or amido-acetic acid), and taurin.

Add a few grains of white sugar, or a drop or two of syrup, to a solution of bile in a test-tube, shake well, add strong sulphuric acid, and cool. A reddish-purple colour is produced at the junction of the two fluids which gradually mounts upwards. This colour appears to be due to the action of furfur-aldehyde, a body formed by

* This so-called mucin is not a compound of a carbohydrate and a proteid, as the true mucins are. It is partially soluble in acetic acid, and gives a nuclein residue on digestion.

the mixture of the sugar and the sulphuric acid, upon cholalic acid.

The test may be done in the following manner: A drop of recently made syrup is mixed with about a drachm of bile, and the mixture is then poured into a porcelain capsule, rinsed round it, and poured out; the adhering trace is dried in the capsule. The capsule is then washed with dilute sulphuric acid, and allowed to drain. On drying, the characteristic colour of Pettenkofer's reaction is developed.

(vi.) To another portion of the filtrate from iii. add chloroform and warm slightly. This extracts the colouring matter (*bilirubin*). Remove the chloroform solution with a pipette and examine it with spectroscope. Observe: *absence of absorption bands*. Apply Gmelin's test.

(vii.) Bile shaken up with oil produces an emulsion. This can under very slight pressure pass through animal membranes; oil alone cannot.

(viii.) Take two filters, wet the one with water, the other with bile, and pour a small and equal amount of oil on each. Oil passes slowly through the one wetted with bile, not at all through the other.

(ix.) Bile precipitates pepsin and peptones. Add a considerable quantity of bile to a solution of fibrin digested in artificial gastric juice (p. 234); a precipitate of pepsin and peptone will fall, and the solution will be found to have lost its proteolytic properties.

Bile Acids, Glycocholic ($C_{26}H_{43}NO_6$), and Taurocholic ($C_{26}H_{45}NSO_7$).

Preparation.—Mix ox-bile, which has been evaporated to one-fourth of its bulk, with animal charcoal, evaporate to perfect dryness on a water-bath, and extract it, whilst still warm, with absolute alcohol. The alcoholic filtrate should be colourless; if this is not the case, more charcoal must be added. The alcohol is distilled off, and the dry residue is again treated with absolute alcohol. The alcohol is then filtered off, and to the filtrate anhydrous ether is added as long as a precipitate is thrown down. The solution and precipitate are to be set aside in a closely-stoppered bottle for some days, when crystals of *bilin* will be produced. If the reagents are not perfectly anhydrous, a gelatinous mass will be formed, but no crystals. Bilin consists of glycocholic and taurocholic acids, which may be separated from one another by dissolving it in water, and adding first solution of neutral lead acetate, and then a little basic

lead acetate. This combines with glycocholic acid to form an insoluble lead glycocholate. Filter, and add to the filtrate basic lead acetate and ammonia; a precipitate of lead taurocholate will be formed, which may be filtered off. In either case the lead must be got rid of by suspending or dissolving in hot alcohol, adding hydrogen sulphide, filtering, and adding water.

Cholesterin.—It is contained in bile, gall-stones, nervous matter, etc.

Preparation. — Cholesterin is usually obtained by extracting powdered gall-stones with ether or boiling alcohol. It crystallizes out on evaporation of the extract. The insoluble residue consists of bile, colouring matter, and mucin.

Tests.—Cholesterin crystallizes in rhombic plates. Examine some of the crystals under the microscope.

It is insoluble in water and cold alcohol; but freely soluble in boiling alcohol and in ether.

It gives a red colour with strong sulphuric acid and with nitric acid and ammonia.

It gives a greenish-blue to violet with sulphuric acid and iodine or zinc chloride; with sulphuric acid and chloroform a play of colours, beginning with blood-red, and ending with green, afterwards disappearing.

Gall Stones:

(i.) Make an ethereal extract. Allow a few drops to evaporate on a slide, and examine for cholesterin crystals with a microscope.

To a portion of the extract add strong sulphuric acid; a bright red colour indicates cholesterin.

(ii.) Boil the residue (coloured) in water with a few drops of acid; pour off the water, and add some warm chloroform.

Examine the chloroform solution for *bilirubin* as above.

CHAPTER VIII.

MUSCLE.

Muscle plasma may be prepared from living muscle by rapidly stripping the muscles, freed as much as possible from blood, from recently killed frogs, and throwing the flesh into a crucible placed on a freezing mixture of pounded ice and salt. When the muscle is thoroughly frozen, it should be brayed in a mortar, then tied up in a piece of linen and squeezed forcibly. A slightly yellow alkaline fluid exudes as the muscle thaws. This is muscle plasma. It coagulates spontaneously to form a clot, which is **myosin** (p. 192) and **muscle serum.**

Muscle plasma may also be obtained by making an extract of living muscle with neutral saline solution, as in the case of blood plasma (p. 221). This is **salted muscle plasma.**

* (i.) Take 10 cc. salted muscle plasma, add to it 50 cc. of distilled water, keep at a temperature of 35° to 40° C. for one or more hours, the diluted solution clots separating out into myosin and salted muscle plasma. Separate by filtration.

(ii.) Dissolve the clot of myosin in 5 per cent. solution of magnesium sulphate, heat to 47° C., a precipitate of **paramyosinogen**; filter, and heat the filtrate to 56° C.; a precipitate of **myosinogen** will occur.

(iii.) Take some salted muscle plasma, and saturate it with magnesium sulphate or sodium chloride; there is a precipitate of **myo-globulin**, which evaporates at 63° C. Filter; heat the filtrate to 73° C.; a precipitate of myo-albumin. Filter. The filtrate contains **myo-albumose** (p. 189).

* Method slightly modified from Halliburton.

(iv.) In muscle serum show the presence of **sarcolactic** or **paralactic acid,** $CH_3CH\begin{matrix}OH\\COOH\end{matrix}$ by the following test:

A purple solution made by taking 10 cc. of a 4 per cent. solution of carbolic acid, 20 cc. of distilled water, and one drop of liquor ferri perchloride, is turned to a yellow colour by the sarcolactic acid.

(v.) Make a solution of Liebig's extract of meat, add solution of lead acetate as long as a precipitate continues to fall. Remove precipitate by filtration, and pass a stream of sulphuretted hydrogen through the filtrate. Remove the lead sulphide by filtration. Concentrate the filtrate, and put aside in a cool place; crystals of **kreatin** will separate out.

CHAPTER IX.

THE DIGESTIVE FLUIDS.

SALIVA.

PROCURE a copious flow of saliva by touching the tongue with a crystal of tartaric acid, avoiding, as much as possible, any mixture of the acid with the saliva. Filter.

1. To show the action of **ptyalin**, the diastasic ferment of saliva, take five test-tubes containing equal quantities of starch solution, and add to each equal quantities of saliva. (i.) Boil briskly and place in the incubator; (ii.) Place in ice and salt; (iii.) Keep at the temperature of the room; (iv.) Place in the incubator; (v.) Render distinctly acid and place in the incubator. At the end of ten minutes test all for sugar by Trommer's test. (i.), (ii.), and (v.) will contain no sugar; (iii.) a little; (iv.) abundance.

2. To show the **inorganic constituents** of saliva, test qualitatively small quantities of the filtered saliva for the following:

(1) *Carbonates.*—Shake with a small quantity of any strong acid. Bubbles of CO_2 will be evolved.

(2) *Chlorides.*—Acidulate with nitric acid, a precipitate with silver nitrate, soluble in ammonia.

(3) *Phosphates.*—A yellow precipitate with ammonium molybdate on boiling.

(4) *Sulphates.*—A precipitate with barium chloride or nitrate, which does not disappear on boiling with nitric acid.

(5) *Potassium.*—A violet colour in the Bunsen flame, visible through blue glass.

(6) *Sodium.*—A yellow flame.

(7) *Calcium.*—Precipitate by ammonium oxalate.

(8) *Magnesium.*—Add, first, ammonium chloride and ammonia, then sodic phosphate; a precipitate of ammonio-magnesium phosphate will result.

(9) *Potassium Sulphocyanate.*—This salt gives a reddish colour on adding ferric chloride, which is not altered by hydrochloric acid, but is discharged by mercuric chloride (meconic and acetic acids give a somewhat similar colour, which is discharged by hydrochloric acid in the former and by mercuric chloride in the latter case).

3. To show the **organic constituents,** test in the following manner:

(1) Acidulate with acetic acid; a precipitate of **mucin.** Filter.
(2) Apply to some of the filtrate the proteid reactions.
(3) Saturate more of the filtrate with solid sodium chloride; a precipitate of **globulin** appears.

GASTRIC JUICE.

Show the digestive action of **pepsin** by means of a glycerine extract of pig's stomach or by means of commercial solid pepsin.

Take three test-tubes containing (i.) hydrochloric acid solution 0·2 per cent. and fibrin; (ii.) pepsin, water, and fibrin; (iii.) pepsin, hydrochloric acid 0·2 per cent. solution, and boiled fibrin; also (iv.) a flask containing pepsin, hydrochloric acid 0·2 per cent., and fibrin, all in larger quantities than above. Place all the tubes in an incubator for fifteen minutes. Note that in (i.) the fibrin swells and becomes transparent, but does not dissolve; in (ii.) and (iii.) the fibrin is unchanged; in (iv.) the fibrin is dissolved. Filter (iv.).

Neutralize the filtrate; a fairly copious precipitate of *syntonin*, *parapeptone*, or *anti-albumose* (Kühne) results. Filter. In the filtrate it is possible, as a rule, unless the ferment is very active, to show the intermediate products of digestion, viz., **proto-** and **deutero-albumoses** (p. 189), as well as that of **true peptone** (p. 190).

Ammonium sulphate precipitates the albumoses, but it has no such effect on peptones.

Reactions of Pure Pepsin:

(1) Should not be precipitated by nitric acid, tannic acid, iodine, or mercuric chloride.
(2) Is precipitated by platinum chloride and neutral lead acetate.

Rennet.—The action of the **rennet** curdling ferment of the stomach upon milk has already been shown.

PANCREATIC JUICE.

The digestive action of the pancreas may be tested by means of extracts of the gland of various kinds in many ways.

Pancreatic extracts may be made by pounding up the pancreas of a pig or dog, after careful removal of the adhering fat, in a mortar, or passing it through a sausage-making machine until in a complete pulp, and then using one of the following solutions as a vehicle for the extraction of the ferments :

(1) Slightly acidulated and very dilute methylated spirit.
(2) Chloroform-water.
(3) Brine.
(4) Glycerine ; or
(5) Water.

After the extract has been made it should be filtered. The gland should have been removed from the body for some hours (up to twenty-four) before the extract is made.

1. Add a few drops of one of the above extracts and some sodium carbonate—1 per cent. solution—to some starch mucilage in a test-tube, expose in a water-bath at 40° C. for fifteen or twenty minutes, test for sugar. The intermediate products (*erythro-dextrin*, giving a red colour with iodine ; and then *achroo-dextrin*, giving no colour with iodine, but precipitated by alcohol) may be tested for, if the action is only allowed to go on for a short time and be stopped by boiling. Experiments proving the conditions under which the diastasic ferment acts best are similar to those described for the salivary ferment ptyalin (p. 233).

2. Place an equal quantity, *e.g.* 2 cc., of several of the above extracts in test-tubes with the alkaline solution as in 1, and add to each a fragment of boiled fibrin ; place them in a water-bath as above for half an hour, neutralize, filter, boil, refilter, and test the filtrate for **peptone**. A considerable difference in the activity of the extracts will be noticed.

3. Proceed as in 2, but boil one solution ; no solution of fibrin takes place in this tube ; allow the unboiled digestive fluid to remain in water-bath for an hour, and watch, from time to time, the gradual erosion of the edges of the fibrin, noting that the fibrin does not swell up and become transparent as in gastric digestion.

4. For intermediate products of pancreatic proteolytic digestion, it is best to employ a considerable amount of fibrin, and a comparatively small proportion of the pancreatic extract. Under these conditions, it is usually possible to demonstrate the presence, as digestion goes on, of **proto-albumose** ; and the resulting solution will probably contain more **deutero-albumose** than true **peptone**, although the latter will be present in greater or lesser amount. If in place of fibrin, gelatin be employed, the resulting **gelatin-**

peptone will be found to react to tests, placing it nearer proteoses than peptone. It is precipitated by ammonium sulphate.

5. Add some of the most active extract to a larger quantity of fibrin and sodium carbonate solution in a beaker to the extent of 2 per cent.; allow the action to go on for two hours at 40° C., then neutralize with acetic acid, filter, boil, and refilter. Evaporate the filtrate to one-third of its bulk, add absolute alcohol while still hot —leave it for twenty-four hours to precipitate peptones—filter, concentrate the filtrate; **tyrosin** will crystallize out: pour off the mother liquid, and concentrate still further; **leucin** will crystallize out.

6. In a pancreatic digestion fluid, when the action has gone on for twenty-four hours at least, notice its smell, and test for **indol**— by acidulating with dilute sulphuric acid—boiling, and adding a drop of very dilute nitrous acid; a reddish-pink colour should appear at once or on standing. If the solution give a purple colour with chlorine water, the associated body, **naphthalamine**, is present.

7. Add some unfiltered watery extract of dog's pancreas to melted lard at 37° C., in proportion of 2 to 1; rub together over a water-bath—a thick creamy emulsion will result.

8. Take some perfectly neutral olive-oil in a test-tube, and add to it about an equal quantity of an unfiltered aqueous extract of dog's pancreas, which should be neutral, or slightly alkaline. Place the test-tube in a warm chamber at 40° C. for five minutes, and test the reaction with litmus-paper; the solution has become acid from the setting free of the fatty acids in the oil.

9. Add some of the brine extract of pancreas to milk in a test-tube at temperature of 40° C.; notice that the casein is soon precipitated and then dissolved, forming **casein peptone**; also in another test-tube do the same experiment with milk diluted $\frac{1}{4}$; note that precipitation does not occur, but that in a few minutes after the addition of the extract the casein can be precipitated on boiling (**metacasein**) as well as on addition of dilute acid.

10. Take four test-tubes and charge them, and in each place pancreatic extract and carbolic acid, 5 per cent., 2 cc.; and in:

(1) Starch solution, 5 cc.
(2) Sodium bicarbonate solution, 5 cc.; a shred or two of fibrin.
(3) Olive oil, 5 cc.
(4) Milk, 5 cc.

Place in incubator at 40° for one hour. Note that in each case the

presence of the carbolic acid has not interefered with the action of the ferments; keep (2) freely exposed to the air for three or four days, and notice that there is no bad smell developed, and no indol produced.

11. In order to test the different activities of the ferments of the pancreatic ferment of different animals, etc., Sir William Roberts has introduced the following very useful plan:

A standard solution of the pancreas is made with dilute spirit in each case, and its diastasic power is calculated from the time which is required for a given quantity of the extract, say 1 cc., to convert a given amount of a standard solution of starch, say 1 per cent.; so that when a drop is added to a drop of a standard iodine solution, say 0·5 per cent., no colour is produced.

And as regards the proteolytic action, a very similar plan is used. A given quantity of the standard solution of the pancreas requires to act upon a given quantity of milk till the metacasein reaction disappears.

THE BILE. See Chap. VI., pp. 228-230.

CHAPTER X.

UNHEALTHY URINE AND CALCULI.

When, in diseased conditions, proteid occurs in the urine, the variety is nearly always chiefly **serum-albumin**; but it should be remembered that **serum-globulin**, in addition, is sufficiently common, and that **egg-albumin** (after an excessive diet of eggs), **proto-** and **deutero-albumoses**, and **peptone**, may all occur, either together or separately. The plan to be adopted in the investigation is shortly as follows:

If the urine is suspected to contain a proteid, apply the xantho-proteic test; if a well-marked orange reaction occurs, proceed to find the variety or varieties thus:

(i.) Slightly acidulate with weak acetic acid and boil; a coagulum will show the presence of serum- or egg-albumin, or of a globulin, or of all three.

(ii.) Saturate the urine with magnesium sulphate after neutralization if a precipitate occurs; it may be globulin or hetero-proteose. If the former, the urine coagulates on heating; if the latter, a precipitate occurs on heating, soluble in dilute acids.

(ii.) If the urine coagulates on heating, but gives *no* precipitate on saturation with magnesium sulphate, the albumin present is serum- or egg-albumin; if the former, it gives no coagulum with ether.

(iv.) If the urine gives the proteid tests, but is not coagulated on heating, it may contain either proto-albumose, deutero-albumose, or peptone. If there is no precipitate on saturation with ammonium sulphate, peptone is present; if there is a precipitate on saturation with ammonium sulphate, but not with magnesium sulphate or sodium chloride, it is deutero-albumose, and not proto-albumose.*

* If all of these proteids be present, which is not at all likely, and it be desired to isolate them, the property which albumoses and peptones possess,

In ordinary cases, the proteids present in albuminuria are serum-albumin and serum-globulin in various proportions—generally speaking, the former in great excess; and we may therefore **in ordinary cases** prove the presence of albumen in such urine in the following manner:

Albumen present.—(i.) If the urine is neutral or acid, the albumen is precipitated on boiling. If alkaline, render slightly acid with nitric acid or acetic acid, and boil. Albumen is precipitated.

Care must be taken, in testing for albumen in urine, that the test-tube which is employed be clean and free from acid. Perform the following experiment to show the necessity of this precaution. To a small quantity of albuminous urine add an excess of strong nitric acid; a precipitate of albumin is thrown down. Pour the contents of the test-tube away, and *without* washing it, fill it up with a fresh sample of the albuminous urine. After allowing it to stand five minutes, boil the solution, when, although albumin is known to be present, no coagulum will be formed, since the acid remaining in the test-tube from the previous experiment has been sufficient to convert the albumin into acid albumin. The experiment will frequently, however, be unsuccessful, unless a large excess of the strong acid has been first added.

(ii.) *A more delicate test.*—Acidify urine with acetic acid; mucus will be precipitated if present. Filter, and add ferrocyanide of potassium to the clear filtrate; a precipitate will be formed in presence of albumin.

(iii.) *Heller's test* (p. 184).

(iv.) Acidulate the urine with citric, tartaric, or acetic acid, and then add to separate portions in test-tube:

(*a*) Saturated solution of picric acid.
(*b*) Saturated solution of sodium tungstate.
(*c*) Solution of potassio-mercuric iodide.
(*d*) Solution of potassio-mercuric iodocyanide.
(*e*) Brine.

In each case the ordinary albumin, even in small amount, will be precipitated.

Bile pigment or acid present.—Adopt tests (p. 228).

Uric acid.—Employ the murexide test (p. 208).

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of not being coagulated by prolonged action of alcohol, while serum-albumin and globulin are, and the remarkable exemption which peptone shows from the rule that proteids are precipitated by saturation with ammonium sulphate may be made use of.

Urates.—The deposit dissolves on heating; sometimes reappears on cooling. Urates dissolve in caustic alkalies; uric acid is separated on adding strong acids. Apply the murexide test to the deposit.

Phosphates.—The phosphates may be in solution, or may form a deposit. If in solution the urine is feebly acid or neutral. On boiling urine, the phosphates are deposited, the deposit being soluble in weak acid. Deposits of phosphates are insoluble in caustic alkalies.

The phosphates are either in the form of amorphous phosphate of lime ($Ca_3(PO_4)_2$), crystallized phosphate of lime ($2CaH,PO_4$), or ammonio-magnesium phosphate or triple phosphate ($Mg(NH_4)PO_4+6H_2O$), sometimes precipitated all together, or the first and third varieties.

The reason why phosphates are deposited on boiling in urine which was before clear is uncertain, but possibly it may be due to the action of heat expelling carbon dioxide, or decomposing urea into ammonium carbonate, which renders the urine alkaline.

Oxalates.—The deposit is soluble in hydrochloric acid, but insoluble in acetic acid. Examine their crystalline form with a microscope.

Sugar (Diabetes).—When sugar is present, the urine is generally light-coloured, with a sweet smell, acid, of high specific gravity—viz., from 1030-1050. The presence of sugar is proved by Trommer's test, or by any other of the sugar tests mentioned (p. 200).

Quantitative estimation:

(1) *By Fehling's Method.—Solution required*=copper sulphate and caustic soda, with some sodic potassic tartrate of such a strength that 10 cc. of solution contain the amount of cupric oxide which ·05 grm. of sugar can reduce to cuprous oxide. (This solution should be freshly prepared. It is made as follows: Take of the sulphate of copper, 40 grms.; neutral tartrate of potash, 160 grms.; caustic soda (sp. gr. 1·12), 750 grms.; add distilled water to 1154·5 cc. Each 10 cc. contains ·05 grm. of sugar.)

Remove any albumen which may be present in the urine by boiling and filtering. Take 10 cc. of the urine, free from albumen, and add 90 cc. of distilled water. Place this in a burette. Put into a flask or dish 10 cc. of the standard solution, and dilute with four times its bulk of water and boil. Run into it, from a burette, some of the diluted urine, say 20 cc., and boil. Allow the precipitate to settle, and if the supernatant fluid is still blue,

add, say, 5 cc. from the burette, and boil again, and so on, till the fluid ceases to have a blue tinge, taking care, towards the end of the process, to add only a few drops each time. If, after adding 20 cc. of diluted urine and boiling, the fluid has been decolourized, too much urine has been added, and another 10 cc. of standard solution must be measured out, running in less than 20 cc. (say 10 cc.) in the first instance.

When the number of cc. of diluted urine required to decolourize the solution has been determined in this way, that volume contains the amount of sugar necessary to reduce 10 cc. of standard solution—*i.e.*, ·05 grm. But one-tenth only of this is urine, ∴ one-tenth of the number of cc. used contains ·05 grm. of sugar. From this the percentage can easily be calculated.

(2) *Pavy's Modification of Fehling's Method.*—By Fehling's method it is difficult and tedious to judge of the point of complete reduction of the cupric oxide. Dr. Pavy, accordingly, uses a strongly ammoniacal solution of the above. A certain amount is introduced into a small flask, which is then heated till the vapour of ammonia escapes by a narrow tube. The sugar solution is then allowed to flow from a burette into the flask until the blueness has disappeared, the solution being kept boiling all the time. The blueness is apt to disappear suddenly, and care should therefore be taken towards the end of the process.

Calculate as in Fehling's method.

(3) **By fermentation.**—Take the specific gravity of the urine before and after fermentation. Each degree of specific gravity lost by the urine represents one grain of sugar per ounce of urine.

(4) Sugar may also be estimated **by adding yeast** to urine, and collecting the carbon dioxide evolved. The carbon dioxide is a measure of the amount of sugar present.

(5) **By the saccharimeter** (p. 201).

Acetone (dimethyl ketone, C_3H_6O) may be detected in urine by its characteristic smell like pineapple. Several tests may be applied, of which the following is good, but not entirely characteristic: It is best to acidulate the urine with hydrochloric acid, and to distil. To the distillate add some solution of iodine in iodide of potassium and caustic soda; a precipitate of iodoform, yellow in colour, will occur. Or if to a dilute alkaline solution of sodium,

nitro-prusside; if acetone be present, the slight red tint is deepened to a ruby-red, which soon becomes yellow.

Blood.—Either blood corpuscles or hæmoglobulin may be present in the urine. In both cases the guaiacum test (p. 216), the spectrum test (p. 217), and the hæmin test (p. 83) apply; but in the latter case blood corpuscles cannot be detected on microscopical examination.

Pus.—Examine the deposit with the microscope; if pus be present, corpuscles practically identical with leucocytes will be seen. To the sediment of the urine add caustic potash; the urine becomes stringy.

Carbolic Acid.—The urine is dark olive-green or black when first passed; on standing a deposit resembling altered blood often takes place, and the urine becomes lighter in colour. On the addition of strong sulphuric acid, the odour of tar is exhaled from the urine. The addition of perchloride of iron develops a blue colouration.

Salicylic acid gives a purple colour with the perchloride of iron.

Chylous Urine.—The urine may be clear or milky when passed. It contains fibrinogen, as on standing it coagulates, forming a tremulous mass, which after a time liquefies. Examine the urine for albumin, molecular fat, and the nematoid worm, *filaria sanguinis hominis*.

Diazo Reaction.—A characteristic reaction may be shown in urine under certain conditions, particularly in the urine of typhoid patients. Two hundred cc. of a concentrated solution of sulphuric acid are mixed with 10 cc. of pure hydrochloric acid, and 6 cc. of a solution of sodium nitrate (1 in 200). A quantity of the mixture is added to an equal amount of the urine, and made strongly alkaline with ammonia. A bright red colouration occurs. On standing, a deposit occurs, the after part of which is green or black.

URINARY CALCULI AND DEPOSITS.

(i.) Ignite a small portion on platinum foil. If it burn away completely, it is probably uric acid. To confirm this apply the murexide test.

(ii.) Boil the powdered calculus with distilled water, or, if a urinary deposit, with the supernatant urine.

The powder or deposit may be *dissolved* wholly or partially—or it may remain *undissolved.*

(A) The **dissolved** portion consists of

Urates—which are mostly deposited on cooling.

Test for urate of ammonium by boiling with potash to demonstrate the presence of ammonia; and by the murexide test for the uric acid. If it is not ammonium urate, it is probably potassium or sodium urate; for either base, test in the ordinary manner.

(B) The **undissolved** portion may consist of

Phosphates,
Calcium oxalate,
Uric acid.

(iii.) Take some of (B), add a few drops of hydrochloric acid, and boil.

(C) **Dissolved.**
Phosphates,
Calcium oxalate.

(D) **Undissolved.**
Uric acid.
Confirm by the murexide test.

(iv.) Take some of (C) solution and add excess of ammonia; a precipitate will fall in either case: add acetic acid in excess; the precipitate is

(E) **Dissolved.**
Phosphates.
Confirm by the molybdate test.

(F) **Undissolved.**
Oxalates.

If the precipitate in (C) solution is partially dissolved and partially undissolved, phosphates and oxalates are probably mixed.

TABLE OF ABNORMAL URINES.

Urines may be **abnormal** in:

(i.) **Colour**:

Blood (red or smoky), *carbolic acid* (black). *Bile* (brown), *cystin* (yellowish green). } Too dark.

Albuminous urine. *Chylous* ,, } Too light.

Excess of nitrogenous constituents (orange).

Effects of drugs—e.g. rhubarb, red.

(ii.) **Smell**:

Sweet in diabetic urine.

Very rank in urine containing excess of urea or urates.

Ammoniacal in decomposing urine.

Sweetbriar, when *cystin* is present.

(iii.) **Reaction**:

Alkaline, with excess of phosphates.

Strongly acid, with urates in excess.

(iv.) **Specific Gravity**:

Too high in diabetes millitus, and in excess of urea.

Too low in chronic Bright's disease, hysteria, and anæmia.

Abnormal urines may

(A) **Contain no sediment on standing.**

Albumen (Proteiduria).
Phosphates.
Sugar.
Bile.
Carbolic acid.
Blood.
Salicylic acid.

(B) **Contain sediment on standing.**

Urates.
Phosphates (sometimes).
Pus.
Mucus.
Oxalates.
Uric acid.
Albumen (Proteiduria).
Carbolic acid (sometimes).
Cystin.
Chylous urine (becomes transparent when shaken up with ether; test for albumen).

(A) If containing no sediment.

Boil.

Precipitated.	Not precipitated.
Serum-albumin. *Serum-globulin.* *Hetero-albumose.* *Phosphates.* *Blood.*	*Sugar.* *Bile.* *Carbolic acid.* *Salicylic acid.* *Proteose* (p. 189). *Peptone* (p. 190).

Add nitric acid.

Precipitate dissolved.	Undissolved.
Phosphates. *Hetero-albumin.*	*Serum-albumin and Serum-globulin.* *Blood.*

With fresh sample of urine apply guaiacum test (p. 216).

Add to some of the urine caustic soda.

Precipitated.	Not precipitated.
Phosphates.	*Hetero-albumose.*

Blue colour.	No Colouration.
Blood.	*Serum-albumin and Serum-globulin.*

Examine with the microscope.

Apply Gmelin's test (p. 228).

Play of colours.	No play of colours.
Bile.	*Sugar.* *Carbolic acid.* *Salicylic acid.*

Apply Trommer's test (p. 200).

Reduction of copper.	No reduction.
Sugar.	*Carbolic acid.** *Salicylic acid.**

* *Apply special tests* (p. 242).

(B) **If containing a Sediment:**

Boil.

Precipitate or sediment dissolved.	Sediment undissolved.
	Phosphates—increased.
	Albumin (*Serum-albumin and Serum-globulin*)—increased.
Urates.	*Pus.* *Cystin.*
	Mucus.
	Oxalates.
	Uric acid.
	Carbolic acid (see above).

Add nitric acid.

Precipitate dissolved.	Precipitate undissolved.
Phosphates (soluble in acetic acid).	*Albumen*—increased.
Oxalates (insoluble in acetic acid. Examine sediment with microscope, octahedral crystals or dumb-bells).	*Pus.*
	Mucus—not increased.
Cystin (do. hexagonal plates).	*Uric acid.*

Add caustic potash to fresh portion.

Dissolved.	Undissolved.
Uric acid.	*Albumin.*
Pus (converted into a glairy mass).	
Mucus.	
Apply confirmatory tests.	

CHAPTER XI.

EXAMINATION OF ORGANIC SUBSTANCES.

The following notes may be useful. They are not intended to be exhaustive, and the advanced student is advised to supplement them by observations of his own.

(I.) PROTEIDS.

Plan of examination of a solution containing one or more proteids, with and without other substances.

1. Notice whether clear; if not, filter.

2. To the filtrate or to the original solution apply the proteid reactions, with nitric acid, with Millon's reagent, with copper sulphate and caustic potash.

3. Try the reaction of the filtrate or original solution:

May be (*a*) acid,
(*b*) alkaline,
(*c*) neutral.

If (*a*) or (*b*) carefully neutralize:

A precipitate indicates *acid* or *alkali-albumin*, as the case may be, if, when re-dissolved in dilute acid or alkali, after filtration, the solution gives the proteid reactions.

4. Boil some of the original solution. A precipitate indicates (*d*) a *native-albumin*, or (*e*) a *globulin*.

To distinguish (*d*) and (*e*):

5. Saturate a portion of original solution with solid sodium chloride or magnesium sulphate.

Precipitated globulin	*Non-precipitated native-albumin*
(Pour some of original solution into distilled water; a distinct precipitate shows [derived-albumins having been excluded] that *myosin* is present).	(Apply special tests for *serum* and *egg-albumin*).

If the presence of native-albumin as well as globulin be suspected, filter, and boil the filtrate—coagulation proves it, unless it be slight, when it may be due to imperfect precipitation and separation of globulin. If in doubt, saturate the filtrate, after the precipitation and removal of the globulin, with sodium sulphate; a precipitate will occur if native-albumin be present.

6. To filtrate from 3, if proteoses or peptone be suspected as well as acid- or alkali-albumin, or to filtrate from 4 if it is suspected as well as native-albumin or globulin, saturate the solution with ammonium sulphate first of all, remove any precipitate of proteose (p. 189) by filtration, and test filtrate for peptone with biuret test, etc. (p. 190). Remember that gelatin gives almost exactly similar tests as peptone, and so put aside some of the solution to cool, and if gelatin be present in sufficient amount, it will solidify; then proceed as in III. 2.

A solution which has been proved to contain one or more proteids may yet contain other organic substances, and to detect these, it is necessary to remove the proteids as far as possible by the above methods—saturation with ammonium sulphate will remove all but peptone—and to apply special tests according to II.

If in filtration of the original fluid in 1, a considerable amount remains on the filter, proceed as in IV.

(II.) CARBOHYDRATES.

A solution from which the whole of the proteids, except peptone, has been removed, by boiling with sodium sulphate and acetic acid and filtering, or by saturation with ammonium sulphate, or which is known to contain no proteids, may be examined for carbohydrates as follows:

1. Add *iodine solution*.

A *blue* colouration, which disappears on heating, and reappears on cooling = *starch*.

A *port-wine* colouration = *glycogen* or *dextrin* (refer to p. 199 for differences).

2. If peptones be present, remove them by the following method: Carefully evaporate the solution to dryness, and add boiling absolute alcohol. Separate the solution from the residue by decantation and filtration. Evaporate off the spirit. Make a watery solution of the residue and test for *sugar*.

3. If no peptones be present, apply Trommer's test for *sugar*.

4. If starch or sugar be present in the solution, and if glycogen or dextrin be suspected as well, it becomes necessary to add to a small portion in a test-tube rather more than its bulk of absolute alcohol. A precipitate of glycogen, which may be redissolved in water and tested with iodine, will occur if it be present. Concentrate the filtrate and add an excess of absolute alcohol. If dextrin be present it will be precipitated.

Other organic substances may still be present. Proceed as in the succeeding sections.

(III.) GELATIN, MUCIN, UREA, URIC ACID.

1. Proceed as in I. If the solution give the xanthoproteic and Millon's tests, but none of the other proteid tests, *mucin* or *chondrin* (*tyrosin*) may be present.

Add some dilute acetic acid to a portion of the solution:

Precipitate = *mucin* or *chondrin*.

Confirm by obtaining a precipitate with alcohol,

No precipitate with tannic acid,
No precipitate with perchloride of mercury.

Mucin and *chondrin* cannot be distinguished from each other.

2. If the solution give the above proteid tests, and also the biuret (purple) reaction, but there be no reaction with ferrocyanide of potassium and acetic acid, and also no precipitate with acetic acid alone, test for *gelatin* (p. 194), and allow the solution to cool; it will gelatinize.

(If tyrosin be suspected, extract with a few drops of hot dilute ammonia and evaporate a portion to dryness; allow it to crystallize, and examine with a microscope.)

3. If no reaction has resulted from the methods given in 1 and 2 and from the above, the solution may contain *urea* or *uric acid* (in the form of urates).

(i.) To some of the solution add sodium chloride and then nitrate of mercury drop by drop. If no precipitate forms at first, but afterwards appears, *urea* is probably present.

(ii.) Confirm by obtaining crystals of *urea* and of *urea nitrate*, and examining with microscope.

(iii.) Add to some of the solution in a test-tube some pure nitric acid, and to another portion in a second test-tube some fuming nitric acid; if the second tube shows brisk effervescence, and the first tube is unaffected, urea is present.

4. Evaporate some of the solution and apply the *murexide test* for *uric acid*.

(If uric acid be suspected it is better to acidulate some of the solution with hydrogen chloride, and allow it to stand for twenty-four hours. Uric acid, if present, falls as a crystalline deposit, and may be examined microscopically, or by the murexide test.)

(IV.) EXAMINATION OF A SOLID SUBSTANCE.

1. *Preliminary Examination.*—Observe whether it be amorphous or crystalline (if the latter, examine the crystalline form under the microscope), and apply the xanthoproteic, biuret, and ferrocyanide of potassium and acetic acid reactions. Observe its odour, if any. Thus gain a clue as to the nature of the substance. Then proceed as follows:

IF A PROTEID OR GELATIN:

2. Test its solubility in water.

SOLUBLE.	INSOLUBLE.
Native-Albumins.	*Other proteids.*
Proto- and Deutero-Albumoses.	
Peptones.	
Gelatin.	

If soluble, apply the special tests to the solution; if not, try its solubility in acid, alkali, and salt solutions, and then apply the special tests.

IF NOT A PROTEID:

3. Test its solubility in water (COLD).

SOLUBLE.	INSOLUBLE.
Grape-sugar.	*Starch* (soluble in HOT water).
Milk-sugar.	*Tyrosin* (soluble in HOT water).
Inosit.	*Uric acid.*
Glycogen.	*Cholesterin.*
Dextrin.	(*Fats.*)
Urea.	
Leucin.	

4. If not a proteid, and soluble in water, add iodine solution to a portion of the watery solution.

Port-wine colouration = *glycogen* or *dextrin*.

Distinguish glycogen and dextrin by the odour and by their respective solubility in alcohol.

5. If there is no result with 4, apply Trommer's test to a portion of the watery solution. If cupric oxide is reduced = *grape-sugar*. *milk-sugar*.

Distinguish by adding absolute alcohol to a portion of the solution (after concentration).

Milk-sugar, white precipitate.
Grape-sugar, no precipitate.

6. If there is no result with 4 or 5, apply the special tests for *urea*.

7. If there is no result with 6, apply the special test for *leucin* as follows:

Heat in a dry test-tube in Bunsen's flame; a smell of amylamine = *leucin*.

(8. If there is no result, apply the special test for *inosit* as follows:

Add nitric acid to original substance, evaporate carefully, moisten with a solution of calcium chloride; evaporate again, *a rosy-red spot* = *inosit*.)

9. If not a proteid, and not soluble in cold water, apply heat:

SOLUBLE.	INSOLUBLE.	MELT.
Starch.	*Uric acid.*	*Fats* (apply the ether test).
Tyrosin.	*Cholesterin.*	
Urates.		

If soluble, to a portion of the watery solution apply the test for *starch*. If no result, test for *tyrosin* (see 3), and for *urates* (p. 240).

10. If not a proteid, and insoluble in water (cold or hot), apply murexide test for *uric acid*.

11. If no result, test for *cholesterin*.

PART III.

PRACTICAL PHYSIOLOGY.

CHAPTER I.

THE CIRCULATION.

The circulation may be studied:

(*a*) In the web of a frog's foot (this is the most easy method, as a frog can readily be obtained at all seasons of the year).

(*b*) In the tail of a tadpole.

(*c*) In the caudal fin of a small fish—*e.g.*, goldfish or minnow.

(*d*) In the mesentery of any of the smaller mammals, such as the mouse or young rat.

To demonstrate the circulation in the web of a frog's foot, prepare a small stand upon which to rest the body of the animal. Such a stand may be readily made by supporting a thin and flat piece of wood covered with cork upon the stage of the microscope, and clamping it firmly in position. A hole with a diameter of about three-fifths of an inch should be made to correspond with the aperture in the microscope stage. At the end of the board nearest the hole, slits are to be cut for the passage of threads. A light-coloured frog is then to be selected, and its head wrapped in a damp cloth, whilst its body is arranged in such a manner upon the stand that one hind-foot extends over the hole in the board, the other being tucked up out of the way. Ligatures should then be passed over the ends of two adjoining toes, and pulled tight. In this way, by a little manipulation, the threads may be fixed so as to allow of a flat surface of the web between the toes being satisfactorily examined. Care must be taken not to stretch the web to an excessive extent, lest the circulation be impeded. In the majority of cases, the frog will remain perfectly quiet for a long period of time, and this is especially the case if the nose of the animal be brought into close contact with the board upon which it lies. Occasionally the frog resists all blandishments of this nature, and exhibits the greatest restlessness. It will then be necessary to subject it to the influence

of ether, or to inject beneath the skin of the back a very weak aqueous solution of urari. The effect of the drug is to render the animal motionless, by paralyzing the endings of the nerves in muscles, and thus preventing the transmission of motor impulses. Urari requires from fifteen to thirty minutes to produce its full effect. The web, after a suitable piece has been obtained for examination, should be brought into focus, and should be examined first with a low power of the microscope, and afterwards with a high power. From time to time, during the examination, the web should be moistened with water. The examination should not be commenced until two or three minutes after the web has been fixed, in order to allow the circulation, as far as possible, to return to its normal condition.

Under the low power notice and draw (*a*) the black *pigment cells* of irregular shape and of varying size lying more superficial than (*b*) *the arterioles*, in which the blood current is more rapid than in (*c*) *the venules*, (*d*) *the capillaries*. Observe the alterations in their

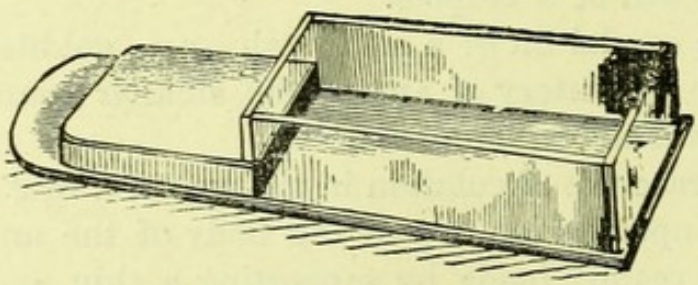

Fig. 70.—Caton's Trough.

size. With the low power select a thin piece of the web, where the vessels can be distinctly seen, and continue the observation under the high power. Determine:

(*a*) The relative positions of the coloured and colourless corpuscles, the coloured in the centre, the colourless at the sides of the vessels.

(*b*) The diapedesis, or passage of colourless blood corpuscles through the walls of the capillaries. This phenomenon can, however, rarely be observed.

The circulation in the tail of the tadpole is readily examined if the animal be first placed in a watch-glass full of water, to which a drop or two of urari solution has been added. When the tadpole has become motionless, it should be transferred to a slide, and examined at leisure. If it is considered necessary, the thinner portions of the tail may be covered with a cover-glass. The same features will be recognised as were described in the case of the frog's web.

For the examination of the circulation in a fish, all that is required

is to place the fish, generally a goldfish, in a suitable vessel, through which a stream of water is kept running continuously. This can be done by means of **Caton's trough** (Fig. 70), or in a simple glass box partially covered over. Into the covered part the tail is inserted, the fish lying comfortably in the trough, which is filled with water, and into which a constant stream flows. The box is placed upon the stage of the microscope, and the tail can be examined with a low power.

The **Sphygmograph** is an instrument used for representing graphically the characters of the pulse. In it a small button rests upon the artery, usually the radial; this button is attached to the

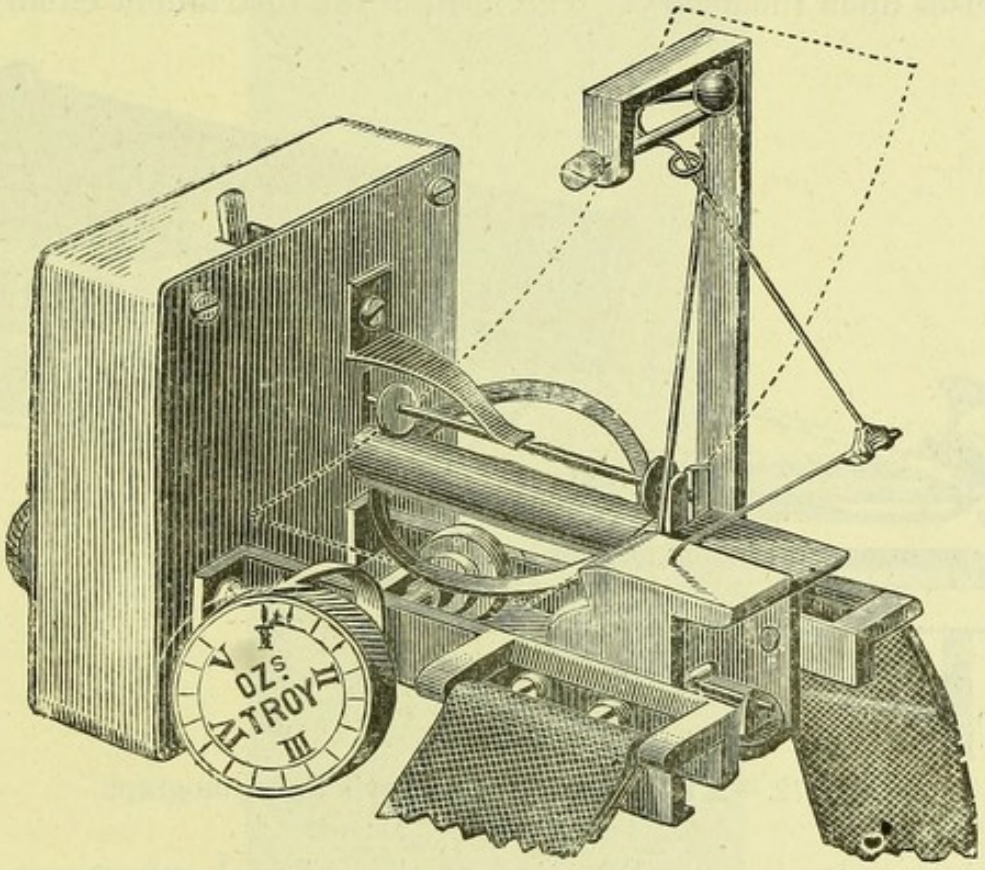

Fig. 71.—Dudgeon's Sphygmograph.

under surface of a steel spring. The movement of the button is communicated to an upright screw working in an arm of metal, which, with the spring, is fixed (although capable of up and down movement) by means of a screw to the frame of the apparatus. The movement of this adjustable screw is communicated to a lever of light wood, since the metal arm has in front a piece of metal projecting upwards, which comes in contact with the lever near its fulcrum. The lever writes on a smoked glass or card, which is moved by clockwork along a groove on the upper surface of the brass box containing the clockwork, which is fixed on the flat piece of metal forming the frame of the instrument. The sphygmograph

is bound on to the wrist with the button on the artery (but not pressing too hard), and the clockwork backwards. The smoked surface is arranged in place; the lever adjusted by means of the screw, so that its end writes on the smoked surface by means of a sharp point. The clockwork is wound up and set going, and the character of the pulse is represented on the moving surface by means of a tracing on the paper. The clockwork is then stopped, the tracing removed, the circumstances under which it was taken noted down, and the paper or glass is varnished. Nearly all the best sphygmographs are provided with an apparatus for approximately adjusting the amount of the pressure which the spring exercises upon the artery. The form of the instrument chiefly used

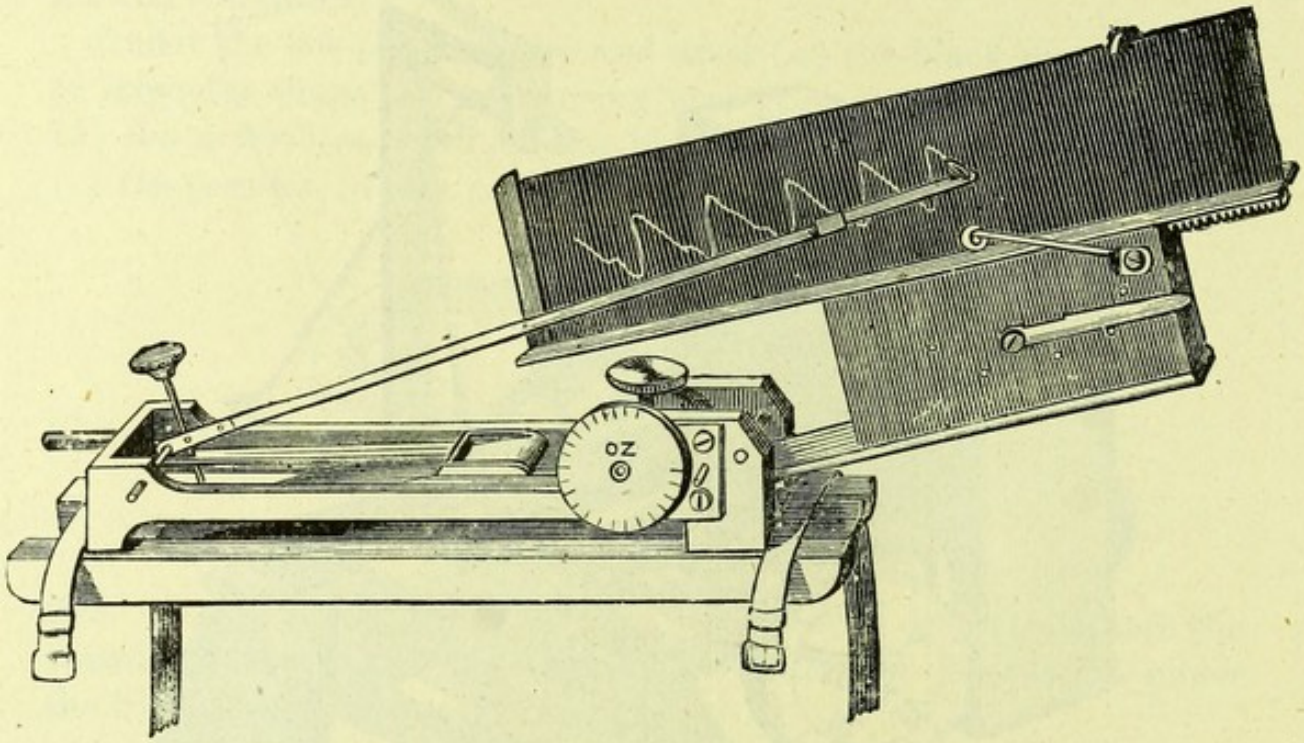

Fig. 72.—A modification of Marey's Sphygmograph.

in this country is a modification of the original sphygmograph of M. Marey.

A small instrument invented by Dudgeon is in considerable use; it is represented in Fig. 71. The advantages claimed for it are: It magnifies the movements of the artery in a uniform degree—viz., fifty times. The pressure of the spring can be regulated from one to five ounces. It requires no wrist-rest, and may be used with equal facility whether the patient is standing, sitting, or lying. With it a tracing of the pulse can be made almost as quickly as the pulse can be felt with the finger. Its sensitiveness is very great, and it records the slightest deviation in the form or character of every beat. Its construction is very simple, and if accidentally broken any watchmaker can repair it. It is very small ($2\frac{1}{2}$ by

2 inches), and light (4 ounces). It is only one-third of the price of the ordinary instruments. It is necessary that the student should make himself thoroughly conversant with the use of the sphygmograph, and opportunities should be taken of practice with both instruments, in both healthy and diseased conditions, and also on the arterial schema.

The arterial schema is an apparatus designed to represent in a diagrammatic form the main phenomena of blood pressure.

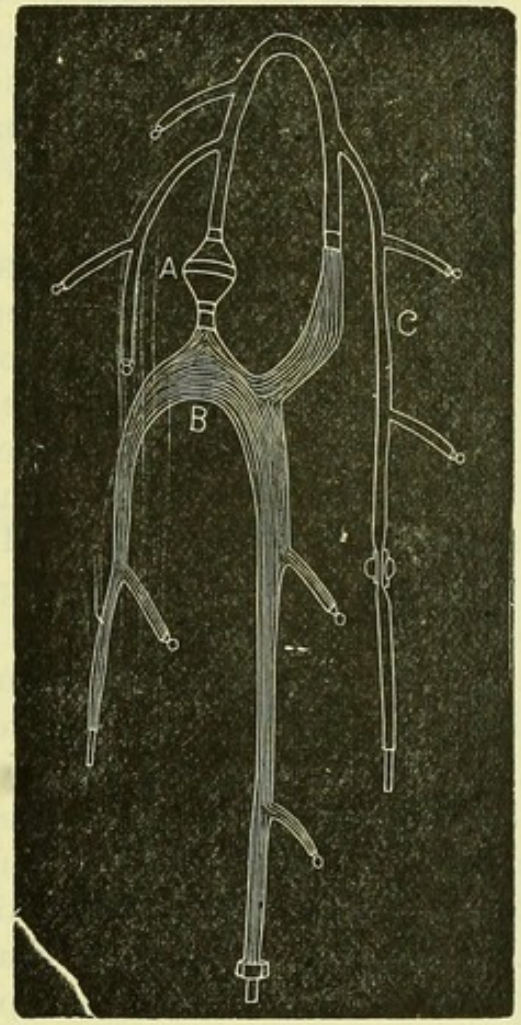

Fig. 73.—Arterial Schema.

A, The heart or pump; B, Flaccid walled vessels or veins; C, Arteries; the end of B communicates with a tap of water. Water may escape at one or two of the arterial tubes, the amount allowed to escape being regulated by clamps.

It consists of a series of elastic tubes of varying calibre, which are arranged somewhat as they are in the vascular system, the larger tubes being equivalent to the main vessels, the smaller ones to the arterioles and venules. The ends of the tubes can be closed by means of ivory pegs, and in this way and by clamps the resistance within the system can be raised to any required extent, and the varying conditions of the circulation can be imitated. In the centre

of the system is a thick-walled elastic sac provided with valves, as in the case of an enema syringe, so that fluid can pass through it in one direction only. When required for use, the tubes are moderately distended with water. Three levers similar to those employed with the sphygmograph are arranged at intervals of six to eight inches along the tubes, in such a way that their movements are recorded in a vertical series of tracings upon the revolving drum. The central sac is then compressed rhythmically by the hand, and the resulting tracings made by the levers are afterwards carefully noted and compared. The apparatus may be also used to show the effect of alteration of the force of the pump or heart, and of the increase of resistance in the tubes caused by their constriction, upon the pressure within them. This may be done by making one of them communicate with a mercurial monometer.

The Estimation of Blood Pressure.*—Make a saturated solution of chloral hydrate in 0·75 per cent. saline solution; inject 15 to 20 minims of the chloral solution beneath the skin of the abdomen of a live rabbit. Leave the rabbit for half an hour, and in the interval prepare the rest of the apparatus. Fill a pressure-bottle with a saturated solution of sodium sulphate or carbonate, and suspend it by means of a string and pulley attached to the ceiling, about four feet above the operating-table. The bottle should have a hole near its lower part, to which a long india-rubber tube is attached; the fluid is prevented from running out by means of a clamp attached to the lower part of the tube. Get ready Czermak's rabbit-holder, and arrange ligatures upon it for binding the fore and hind legs of the animal. Arrange the recording apparatus (p. 264) on one side of the rabbit-holder, and see that the clockwork is wound up, and that it is in working order. Gum a slip of glazed paper round the recording drum, and blacken it by revolving the drum over the flame of a paraffin lamp. The coating of lamp-black should be as uniform as possible, and not too thick. If the kymograph writes with a pencil or with a pen, such blackening is not requisite; in the latter case the can of the pen is filled with a few drops of aniline ink. If a continuous tracing is to be taken, arrange the feeding-roller in a proper position. In the case of the mercurial, or Ludwig's kymograph, see that the U tube is partially filled with clean and bright mercury; that the top of the float is not below the level of the mercury; and that the weighted thread presses upon one arm of the pen in such a way as to keep it in contact with the drum. Take a ⊥-shaped glass tube, connect the

* A license under the Vivisection Act is necessary before this experiment can be legally performed.

vertical portion with the tube descending from the pressure-bottle, or, in place of a pressure-bottle, a syringe full of the saline solution may be attached; and one of the horizontal rami with the tube which projects from the shorter limb of the U tube, by means of a piece of india-rubber or, better still, of substantial leaden piping. To the other horizontal ramus attach a piece of india-rubber tubing,

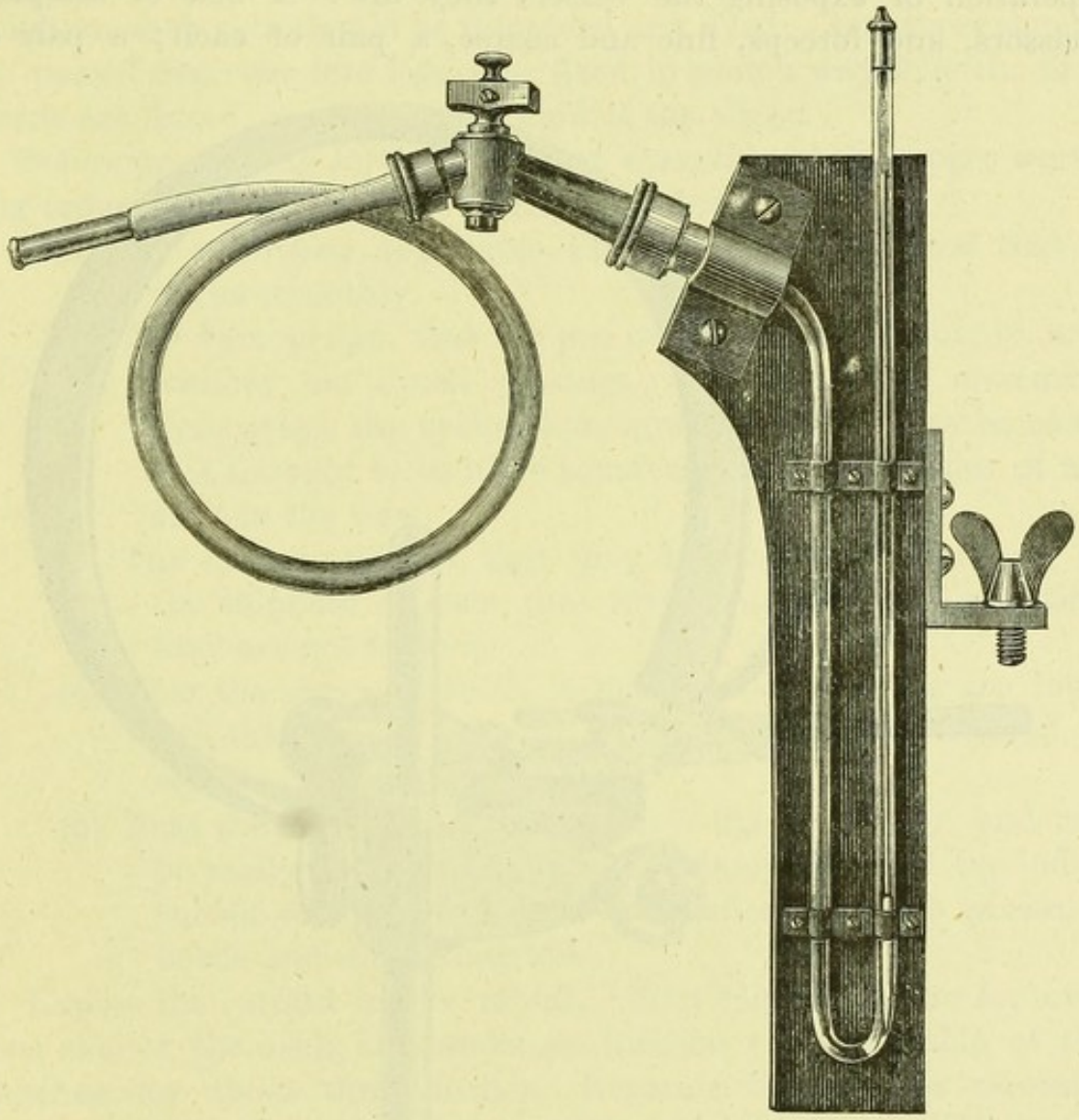

Fig. 74.—Ludwig's Mercurial Kymograph.

The tube to the left of the figure communicates with the artery, and in the part of the U tube to the right is a float which carries a writing style, by means of which the oscillations of the mercury are written upon a recording surface; the whole apparatus being fixed in a convenient position to it by means of a screw clamp to the extreme right of figure.

and clamp the end; open the clamp which has hitherto closed the tube leading from the pressure-bottle; the sodium sulphate will pass through the T-shaped tube, and will fill the tubes in connection with it. Loosen the clip upon the india-rubber tube for an instant, and allow a few drops of the solution to escape; no air should now be

present in the system of tubes thus arranged; if leakage takes place in any part, and air enters, it must be remedied. Select or make a cannula large enough for the carotid or femoral artery of the animal to be experimented upon; this requires considerable experience, and reference had better be made to the demonstrator. Arrange the instruments near at hand which are requisite for the operation of exposing the vessel; they are: A box of scalpels, scissors, and forceps, fine and coarse, a pair of each; a pair of

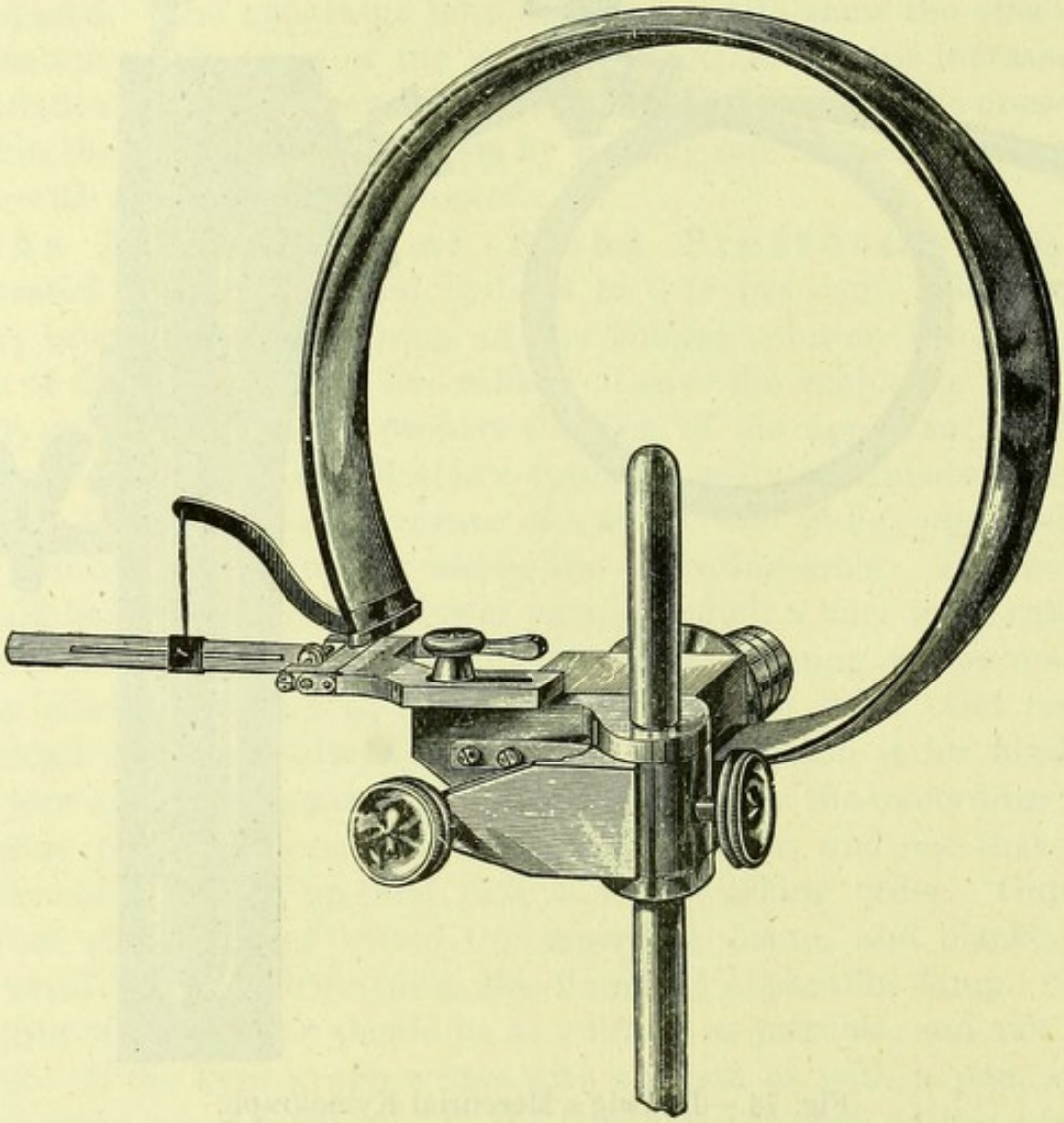

Fig. 75.—Spring Kymograph, a modified form of Fick's instrument.

Krönecker's bull-dog forceps; aneurysm needle; ligatures of silk; a splinter of wood (a match answers the purpose very well); a 'seeker'; and sponges.

Fix the rabbit upon the rabbit-holder; it should be insensible at the time of the operation, and should be fixed by laying it upon its back, unscrewing the bit at the end, and removing the central steel peg; the peg should then be inserted through the side of the bit, in such a way that it passes within the mouth, between the lips and

behind the incisors of the two jaws, passing out on the opposite side, where it fits into a hole in the bit. The animal is thus held by the teeth. The end of the bit should be screwed up; the lower jaw will thus be compressed, and the animal will thereby be prevented from opening its mouth and loosening the central pin; if need be, the pressure may be increased from time to time by means of the screw. The hind-legs should be extended, and the ligatures passed over the thigh on each side should be tightened and fixed Ligatures should be passed over the fore-legs, and fixed in such a way that the first joints are flexed, the limb being held at the elbow.

Before proceeding further, see that everything is in proper working order. The points to be chiefly attended to are:

(1) The *recording apparatus*, that it is wound up, and that it works smoothly.

(2) The *kymograph*, that the pen or style writes distinctly, and without too much pressure; instead of the mercurial kymograph the spring kymograph (Fig. 75) may be used, it is thought to be more accurate; that no bubbles of air exist in the tube.

(3) The *system of tubes*, that they are full of the solution of the sulphate of soda, that they contain no air, and that they are not kinked.

(4) That the *pressure-bottle* is neither too high nor too low; and that if need be it can readily be raised or lowered to equalize the pressure of blood.

(5) That the distal end of the *cannula* fits accurately, and can be easily tied into the proximal clamped end of the india-rubber tubing, which is in connection with the pressure-bottle and the kymograph.

Expose the carotid in the rabbit. First clip away the fur over one side of the neck, and make an incision along the side of the trachea for about three inches. Separate the muscles carefully with a seeker, taking care not to go too far outwards. The artery is readily discovered; it may be distinguished from the large vein which accompanies it by its more opaque appearance, as well as by its lighter colour, and by the pulsation which it exhibits. Dissect it out carefully for a short distance, and pass the aneurysm needle, armed with a ligature, beneath it; withdraw the needle, leaving a loop of thread round the artery; cut the loop, and two ligatures will be thus formed. With very fine bull-dog forceps, clamp the proximal portion—*i.e.*, the part nearest the heart—of the artery. With one of the ligatures tie the distal portion of the artery as high up as the incision will allow. Raise the portion of the artery between

the clamp and the ligature by passing the splinter of wood beneath it, and with a pair of sharp and fine-pointed scissors make a V-shaped incision into it. Fill the cannula, by means of a pipette, with the sodium sulphate solution, and insert it into the incision in the artery; the smaller end being directed towards the heart. Pass the second ligature over the cannula, and tighten it round the artery in such a way as to tie the cannula firmly into the vessel (to facili-

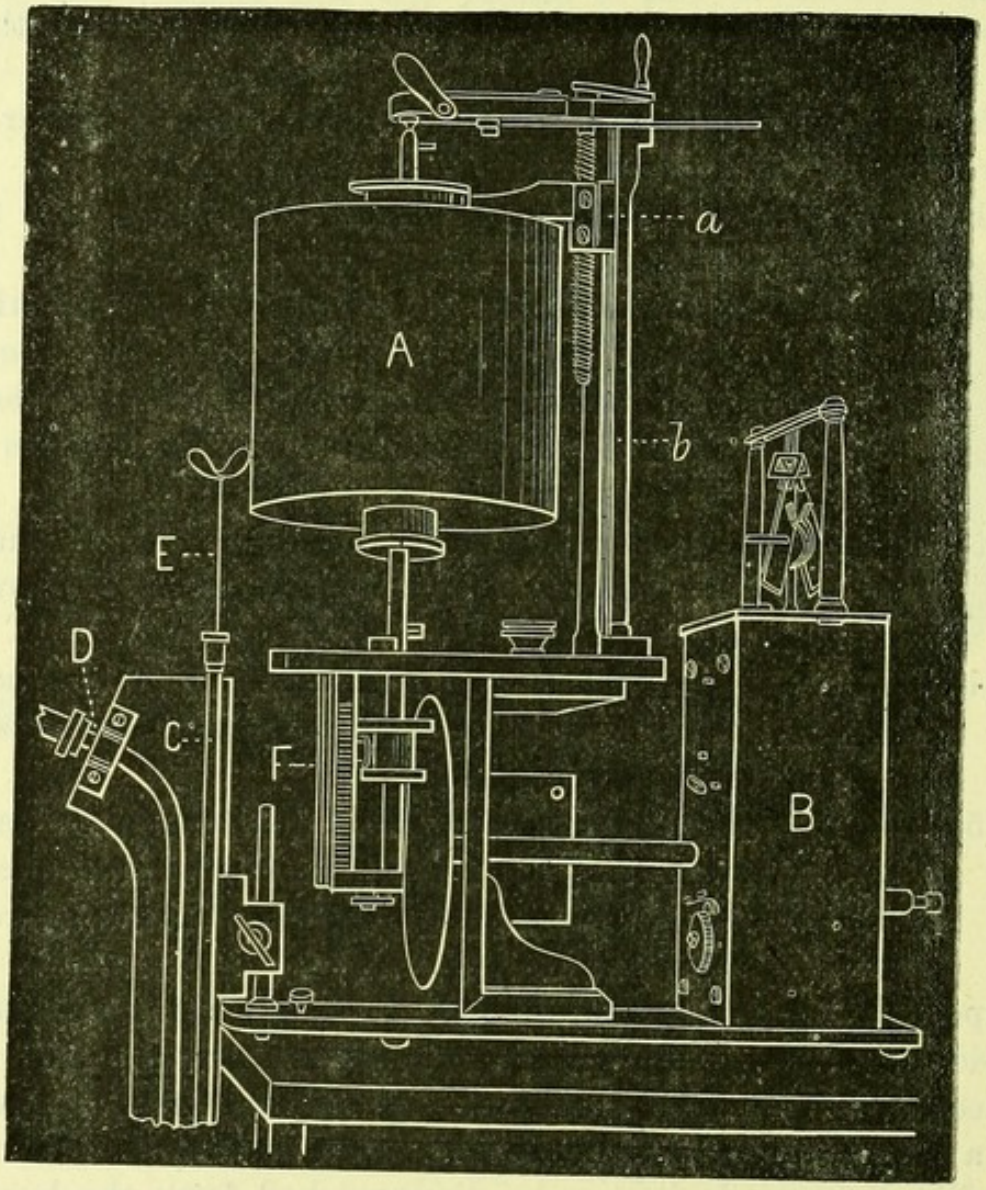

Fig. 76.—Mercurial Kymograph and Recording Apparatus *in situ*.

A, Revolving cylinder, driven by clockwork in B, cylinder supported by an upright, *b*, and capable of being raised or lowered by a screw, *a*, by a handle attached to it, D, C, E, mercurial kymograph.

tate this operation the cannula is provided with a shoulder, behind which the ligature should pass). Fit the india-rubber tube leading through the T-piece to the manometer, on to the other end of the cannula, taking care that both the tube and the cannula are quite full of the soda solution, so that no air may be enclosed at the point

of junction. Remove the clamp which has hitherto prevented the escape of fluid from the india-rubber tube nearest the cannula. Set the clockwork of the recording apparatus in motion; with one hand open *cautiously and gradually* the clamp upon the tube of the pressure-bottle, whilst with the other hand remove the bull-dog forceps from the artery. If everything has been properly arranged, a few drops of blood will pass into the cannula, but the pressure in the artery will be counteracted by the column of sodium sulphate solution, which transmits the variations in the blood pressure to the mercury in the **U** tube, whence it is transmitted to the lever which records it upon the drum. It is possible that the pressure-bottle may be too high, and that the column of liquid is more than enough to counterbalance the blood pressure; in which case the sulphate of soda will enter the blood, and will not only vitiate the experiment, but in many cases will actually kill the animal; hence it is necessary to be cautious in opening the clamp. The sulphate of soda arrests, to a certain extent, the coagulation of the blood, and it is therefore employed; but it often happens that the cannula becomes blocked by a clot, in which case it will be necessary to detach the tubing and to clean out the cannula, or it may be even necessary to clamp the artery, and insert a fresh cannula.

To make a Cannula for use during Blood-pressure Experiments.—Take a piece of hard glass tubing, with a bore of about the size of an ordinary quill pen. Soften the end of the tube in the flame of a blow-pipe, and draw it out gently for about an inch: there will then be a narrower portion of tube between two pieces of the full size. When the tube has cooled, heat a portion of the narrower part in the flame, and draw it out very slightly; by this means the narrower portion will be thicker in the centre than at one side. File through the middle of the narrower portion in an oblique direction. A cannula with an oblique opening at its smaller extremity will thus be formed. It must be finished by carefully rounding off its edges in an ordinary gas flame, and by filing down the aperture with a three-cornered file, until it presents the necessary obliquity. The narrowed portion will have a neck to prevent the ligature slipping off when it is tied into the artery.

The **Cardiograph** registers in a graphic manner the heart's impulse. It consists of two portions: (*a*) A hollow metal disc the face of which is covered with a thin membrane of india-rubber. The disc is often provided with three levelling screws; from its posterior surface passes off a tube bent at right angles. In front of the elastic membrane is an ivory knob, which is in connection with a delicate spring arising from the side of the disc. The extremity of

the spring is also provided with a pointed steel screw, resting exactly on the centre of the membrane. (*b*) The registering portion (Fig. 78, Marey's tambour) consists of a second disc, the elastic membrane of which is in connection with a lever, and from its under-surface a tube also proceeds. The tubes of the two discs are

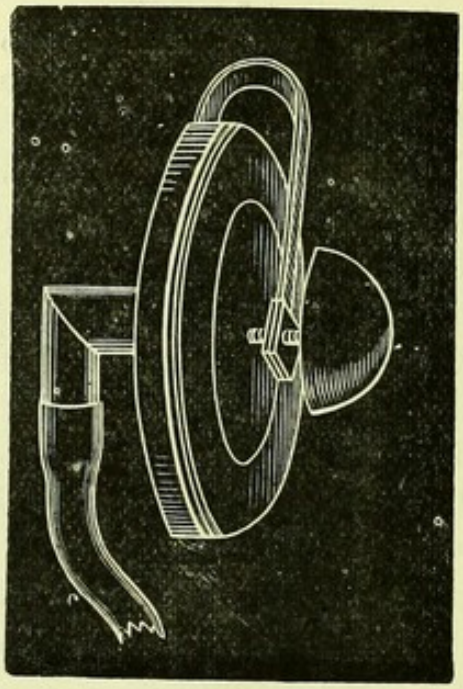

Fig. 77.—Cardiograph.

connected with each other by a piece of elastic tubing, and in this way an air-tight cavity is produced, so that any movements executed by the membrane of the first disc are reproduced in the second disc,

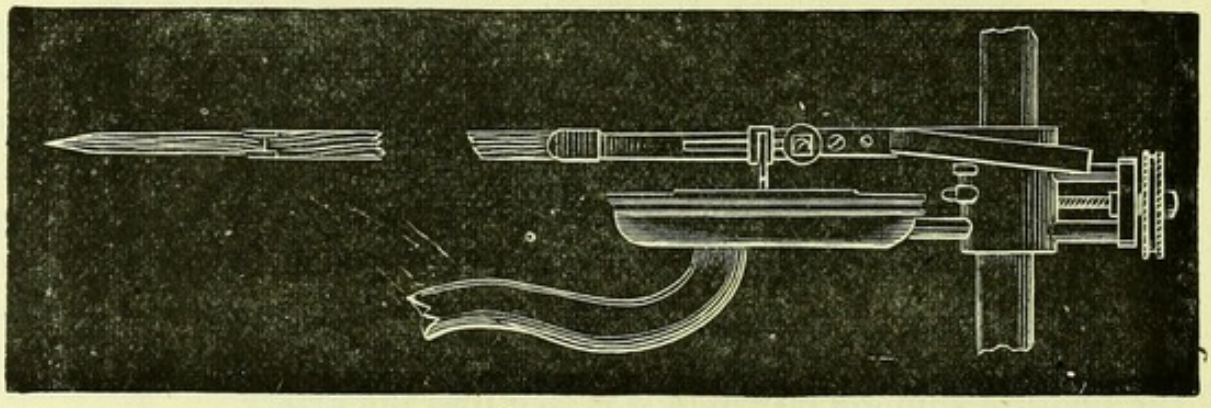

Fig. 78.—Marey's Tambour.

and are transmitted to the lever by which they are recorded in the ordinary way upon the rotating drum. In using the instrument, the patient is made to lie down upon a couch of convenient height; the chest is bared, and the apex beat of the heart is found in the fifth left costal interspace, somewhat below and internal to the nipple.

The first disc is then applied in such a way that the ivory knob is exactly over the point at which the beat of the heart is felt. The impulse is thus transmitted to the lever, which executes certain movements. If these movements be registered upon the revolving drum, it will be found that they consist of a sudden ascent at the instant of the ventricular contraction, and of an equally marked but less sudden fall.

The **Stethoscope.**—The sounds of the heart are heard by means of the stethoscope. The simplest form of this instrument is a cylinder of wood or metal expanded at one end into a conical portion, which is applied to the chest wall, whilst the opposite extremity is provided with a slightly concave disc, to adapt it to the

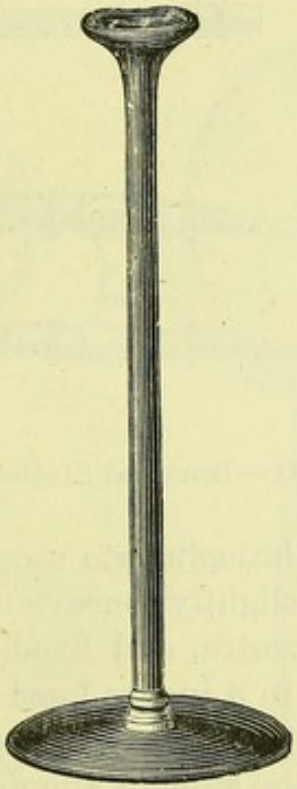

Fig. 79.—Single Stethoscope.

observer's ear. The room should be perfectly quiet, the patient should bare his chest, and remain standing. The finger should then be placed upon the apex-beat of the heart. The observer, standing in front of the patient, should apply the conical end of the stethoscope over this point, and his ear to the opposite end: at the same time he should feel with the fingers of his left hand beneath the sterno-mastoid muscle of the left side for the carotid artery. Two sounds will then be heard, one accompanying the impulse, called the *first* or systolic sound; the other following the impulse, and known as the *second*, or diastolic sound. The first sound is the longer and more deep-toned; it is best heard at the apex of the

heart. The second sound is sharper and shorter; it is best heard in the third intercostal space, close to the sternum, though it is also audible at the apex. After the second sound is a pause, so that the normal cardiac cycle is roughly represented by the rhythm *lubb*, *dŭp* —*lubb*, *dŭp*. In listening to the sounds of the heart, the respiratory sounds may be neglected. Care must be taken that the stethoscope is applied evenly to the chest wall, that the tube is not touched by the clothes or fingers whilst the examination is being made, and that the observer does not press so heavily against the stethoscope as to cause pain to the patient.

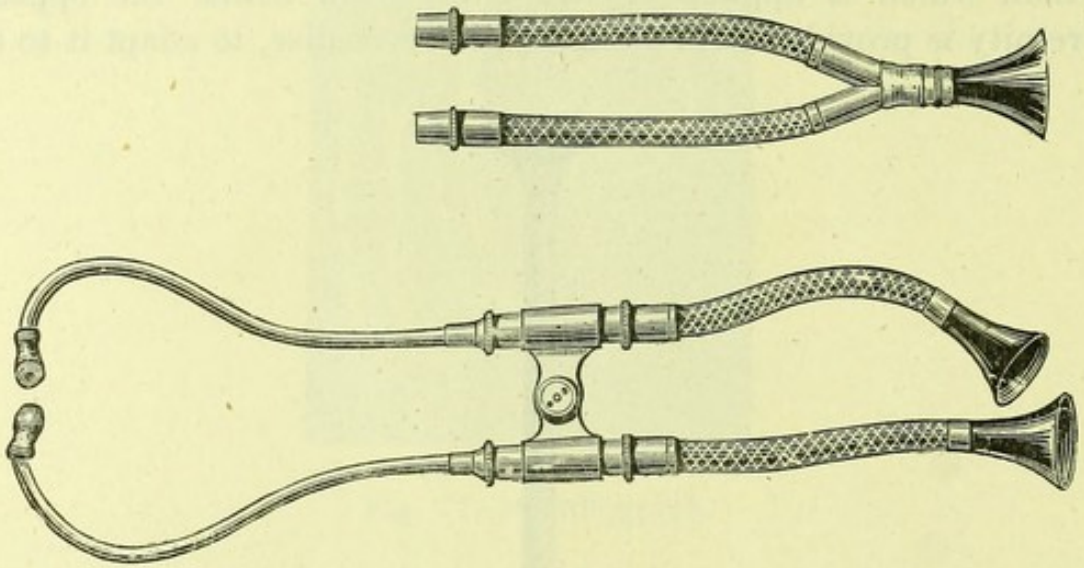

Fig. 80.—Binaural Stethoscope.

The **Ophthalmoscope**, brought into use by Helmholtz, consists in its simplest form of (*a*) a slightly concave mirror of metal or silvered glass, perforated in the centre, and fixed into a handle; and (*b*) a biconvex lens of about 2½ to 3 inches focal length. Two methods of examining the eye with this instrument are in common use—the direct and the indirect; the student should endeavour to accustom himself to use both methods of investigation with equal facility.

A normal eye should be examined; a drop of a solution of atropine* (two grains to the ounce) should be instilled about twenty minutes before the examination is commenced; the ciliary muscle is thereby paralyzed, the power of accommodation is abolished, and the pupil is dilated. This will materially facilitate the examination; but it is quite possible to observe all the details to be presently described without the use of this drug. The room being now darkened, the observer seats himself in front of the person whose eye he is about to examine, placing himself upon a somewhat higher

* Or preferably homatropin hydrobromate, as the effect passes off more rapidly.

level. A brilliant and steady light is placed close to the left ear of the patient. The atropine having been put into the right eye *only* of the patient, this eye alone is examined. Taking the mirror in his right hand, and looking through the central hole, the operator directs a beam of light into the eye of the patient. A red glare, known as *the reflex*, is seen; it is due to the illumination of the retina. The patient is then told to look at the little finger of the observer's right hand as he holds the mirror; to effect this the eye is rotated somewhat inwards, and at the same time the reflex changes from red to a lighter colour, owing to the reflection from the optic disc. The observer now approximates the mirror, and with it his eye, to the eye of the patient, taking care to keep the light fixed upon the pupil, so as not to lose the reflex. At a certain point, which varies with

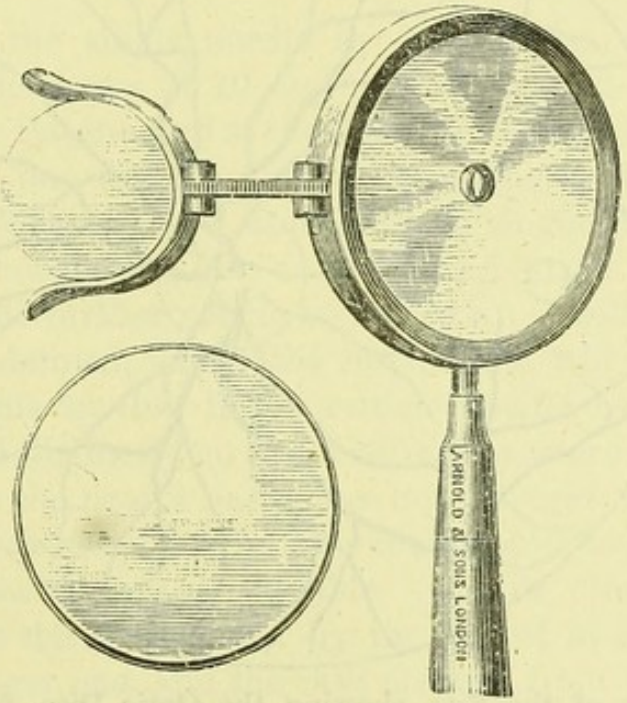

Fig. 81.—Ophthalmoscope.

different eyes, but is usually when there is an interval of about two or three inches between the observed and the observing eye, **the vessels of the retina** will become visible as lines running in different directions. Distinguish the smaller and brighter red arteries from the larger and darker coloured veins. Carefully examine the fundus of the eye, *i.e.*, the red surface—until **the optic disc** is seen; trace its circular outline, and observe the small central white spot, the **physiological pit**: near the centre is the central artery of the retina breaking up upon the disc into branches; veins also are present, and correspond roughly to the course of the arteries. Trace the vessels over the disc on to the retina. The

optic disc is bounded by two delicate rings, the more external being the choroidal, whilst the more internal is the sclerotic opening. Somewhat to the outer side, and only visible after some practice, is the **yellow spot**, with the small lighter-coloured **fovea centralis**, in its centre. This constitutes the direct method of examination; by it the various details of the fundus are seen as they really exist, and it is this method which should be adopted for ordinary use.

If the observer is ametropic, *i.e.*, is myopic or hypermetropic, he will be unable to employ the direct method of examination until he has remedied his defective vision by the use of proper glasses.

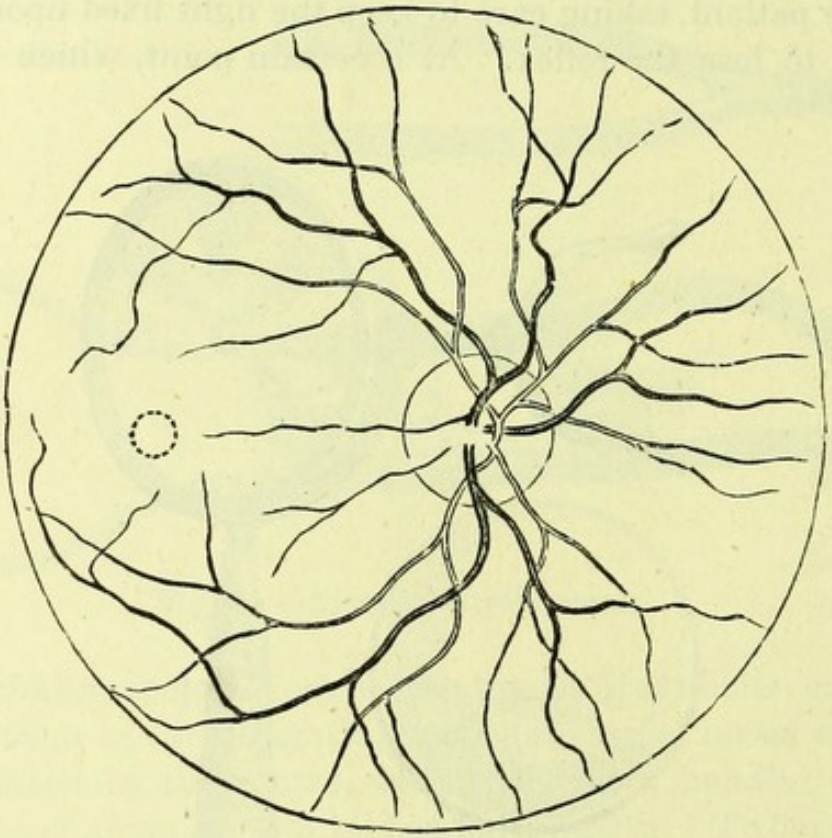

Fig. 82.—Fundus of the Eye, showing the Optic Disc, from which proceed Vessels; those shaded are the Veins, the Arteries are unshaded. The position of the Yellow Spot is indicated by a Small Circle.

In the indirect method the patient is placed as before, and the observer holds the mirror in his right hand at a distance of twelve to eighteen inches from the patient's right eye. At the same time he rests his little finger lightly upon the temple, and holding the lens between his thumb and forefinger, two or three inches in front of the patient's eye, directs the light through the lens into the eye. The red reflex, and subsequently the white one, having been gained, the observer slowly moves his mirror, and with it his eye towards or away from the face of the patient, until the outline of one of the retinal vessels becomes visible, when very slight movements on the

part of the observer will suffice to bring into view the details of the fundus above described, but the image will be an inverted one. The lens should be kept fixed at a distance of 2 to 3 inches, the mirror being alone moved until the disc becomes visible; should the image of the mirror, however, obscure the disc, the lens may be slightly tilted.

Scheiner's Experiment demonstrates the necessity for accommodation, in order that single images may be formed upon the retina. It is performed in the following manner: Prick two minute holes in a card with a needle, taking care that the holes are so near together as to be less than the width of the pupil of the observer's eye, and that they are horizontal. Stick a darning-needle into a long piece of board—a walking-stick answers the purpose excellently—and look at it through the holes; only a single image is seen.

Now replace the single needle by two similar needles, placed respectively at distances of 10 and 20 inches from the end of the board or stick. Put the card at the end of the stick, and look through the holes with one eye, the other eye being closed. Look first at the nearer needle, it will appear to be single and well defined, but the further needle will also be seen as a blurred image; on the other hand, if the further needle be looked at, it will be seen to be single and well defined, whilst the nearer one will appear double. The reason of this is, that if the remote needle be fixed, and its image be formed distinctly on the retina, the more divergent rays proceeding from the nearer needle are not brought to a focus soon enough, but are focussed behind the retina, and as it is looked at through two holes the image is double. On the other hand, if the nearer needle be fixed, the lens, by the act of accommodation, is rendered a stronger one, and the rays coming from a more distant object are brought to a focus in the vitreous, or in front of the retina, and therefore cross. If the nearer needle is looked at, and the right hole in the card be covered, the left-hand image of the double image of the more remote needle disappears, because the rays have crossed in the vitreous. If the more distant needle is fixed and the right-hand hole is covered, the right-hand image of the two images of the near pin vanishes, because the rays have not yet come to a focus.

The **Laryngoscope** is an instrument employed in investigating during life the condition of the pharynx, larynx, and trachea. It consists of a large concave mirror with perforated centre, and of a smaller mirror fixed in a long handle. It is thus used (Fig. 83): The patient is placed in a chair, a good light (argand burner or

lamp) is arranged on one side of his head. The operator fixes the large mirror round his head in such a manner that he looks through the central aperture with one eye. He then seats himself opposite the patient, and so alters the position of the mirror, which is for this purpose provided with a ball-and-socket joint, that a beam of light is reflected on to the lips of the patient.

The patient is now directed to throw his head slightly backwards, and to open his mouth ; the reflection from the mirror lights up the cavity of the mouth, and by a little alteration of the distance between the operator and the patient the point at which the greatest amount of light is reflected by the mirror—in other words, its focal length—is readily discovered. The small mirror fixed in the

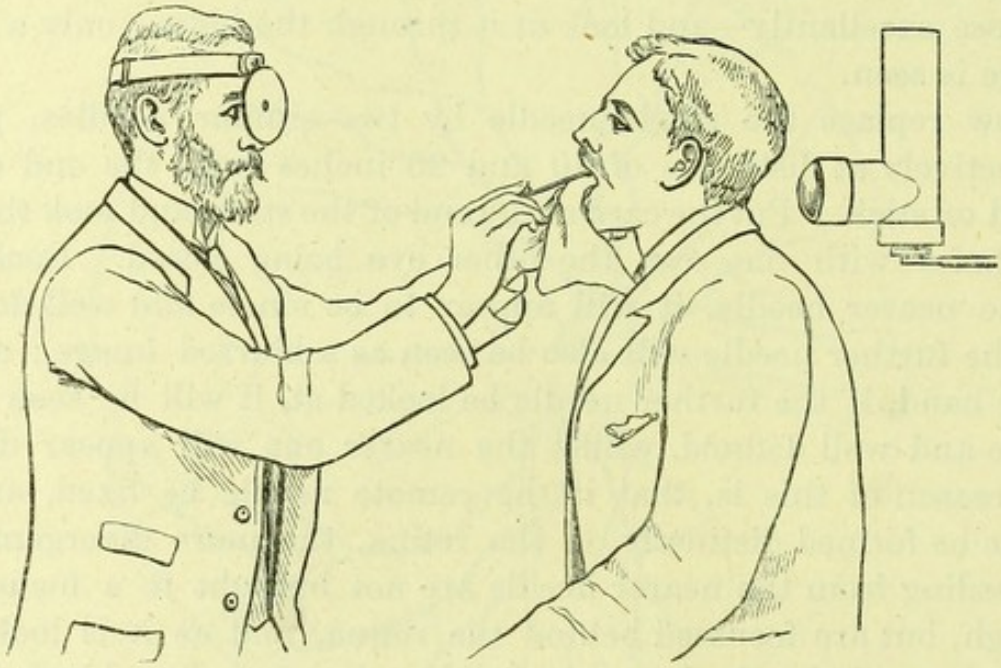

Fig. 83.—To show the position of the operator and patient when using the Laryngoscope.

handle is then warmed, either by holding it over the lamp or by putting it into a vessel of warm water; this is necessary to prevent the condensation of breath upon its surface. The degree of heat is regulated by applying the back of the mirror to the hand or cheek, when it should feel warm without being painful.

After these preliminaries the patient is directed to put out his tongue, which is held by the left hand gently but firmly against the lower teeth, by means of a handkerchief. The warm mirror is passed to the back of the mouth, until it rests upon and slightly raises the base of the uvula, and at the same time the light is directed upon it from the mirror worn by the observer ; an inverted image of the larynx and trachea will be seen in the mirror. If the dorsum of the tongue be alone seen, the handle of the mirror must

be slightly lowered until the larynx comes into view; care should be taken, however, not to move the mirror upon the uvula, as it excites retching. The observation should not be prolonged, but should rather be repeated at short intervals.

The structures seen will vary somewhat according to the condition of the parts as to inspiration, expiration, phonation, etc.;

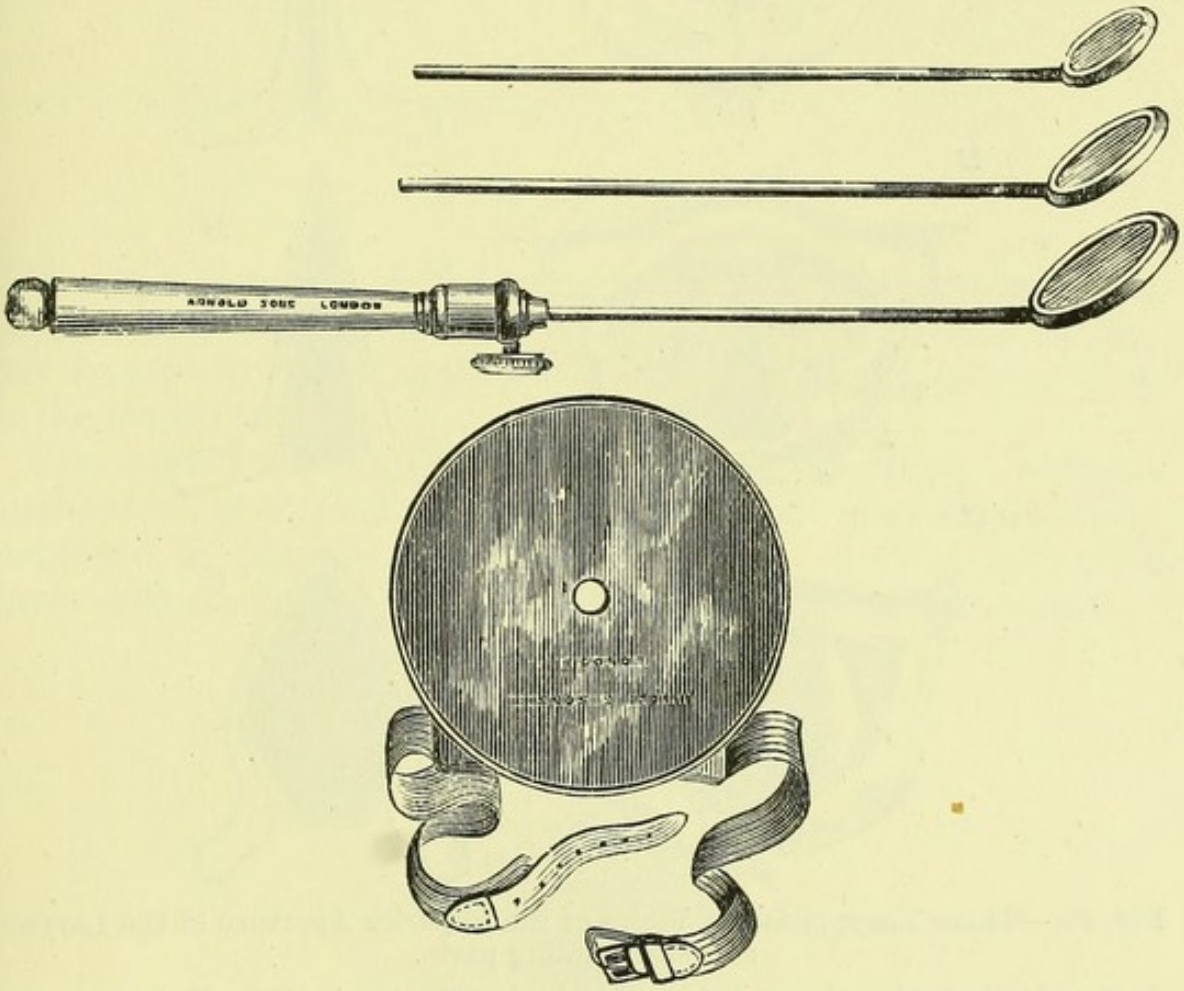

Fig. 84.—The parts of the Laryngoscope.

they are first, and apparently at the posterior part, the *base of the tongue*, immediately below which is the arcuate outline of the *epiglottis*, with its cushion or tubercle. Then are seen in the central line the *true vocal cords*, white and shining in their normal condition. The cords approximate (in the inverted image) posteriorly; between them is left a chink, narrow whilst a high note is being sung, wide during a deep inspiration. On each side of the true vocal cords, and on a higher level, are the pink *false vocal cords*. Still more externally than the false vocal cords is the *aryteno-epiglottidean* fold, in which are situated upon each side three small elevations; of these the most external is the *cartilage of Wrisberg*, the intermediate is the *cartilage of Santorini*, whilst the summit of the

arytenoid cartilage is in front and somewhat below the preceding, being only seen during deep inspiration. The *rings of the trachea*,

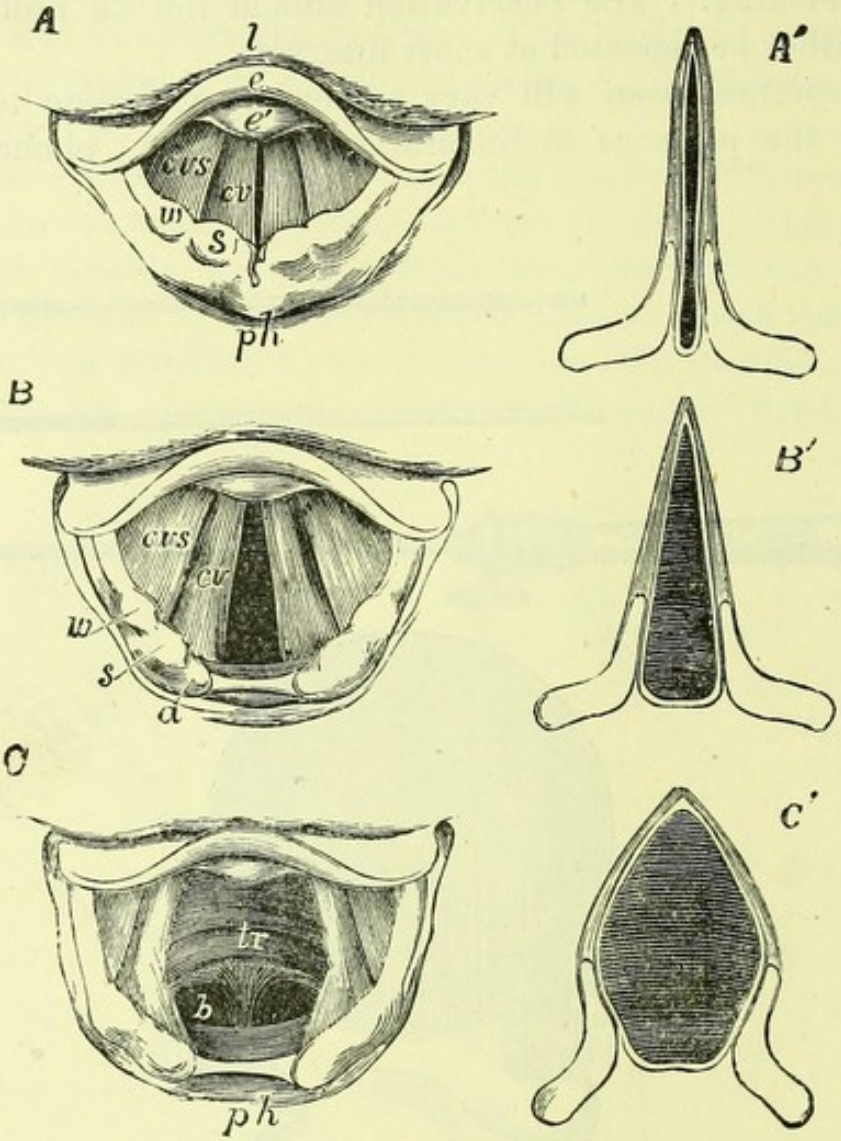

Fig. 85.—Three Laryngoscopic Views of the Superior Aperture of the Larynx and surrounding parts.

A, the glottis during the emission of a high note in singing ; B, in easy and quiet inhalation of air ; C, in the state of widest possible dilatation, as in inhaling a very deep breath. The diagrams A′, B′, and C′, show in horizontal sections of the glottis the position of the vocal ligaments and arytenoid cartilages in the three several states represented in the other figures. In all the figures, so far as marked, the letters indicate the parts as follows, viz. : *l*, the base of the tongue ; *e*, the upper free part of the epiglottis ; *e′*, the tubercle or cushion of the epiglottis ; *ph*, part of the anterior wall of the pharynx behind the larynx ; in the margin of the aryteno-epiglottidean fold *w* marks the swelling of the membrane caused by the cartilages of Wrisberg ; *s*, that of the cartilages of Santorini ; *a*, the tip or summit of the arytenoid cartilages ; *c v*, the true vocal cords or lips of the rima glottidis ; *c v s*, the superior or false vocal cords, between them is the ventricle of the larynx ; in C, *tr* is placed on the anterior wall of the receding trachea, and *b* indicates the commencement of the two bronchi beyond the bifurcation which may be brought into view in this state of extreme dilatation.

and even the bifurcation of the trachea itself, if the patient be directed to draw a deep breath, may be seen in the interval between the true vocal cords.

CHAPTER II.

MUSCLE.

The chief property of living muscle is its **contractility**; this property can only be called forth by means of appropriate stimuli, but the stimuli may be applied as well to the nerve supplying it as to the muscle itself.

The stimuli are of various kinds—either *electrical*, *chemical*, *mechanical*, or *thermal;* but the **electrical** are almost exclusively employed in physiological experiments, and of these two chief forms only are used, viz., the **galvanic** and the **induced** currents.

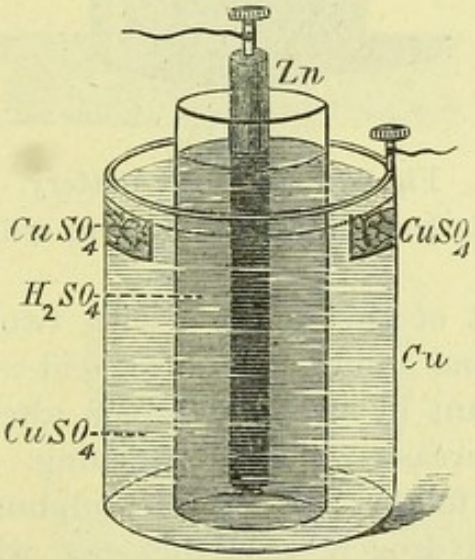

Fig. 86.—Daniell's Battery.

In order to develop and apply the galvanic electrical currents which are used as muscle stimuli in the simplest experiments, certain pieces of apparatus are required, and it will be best to enumerate and describe these first of all, and afterwards to give an account of those which are more complicated.

Batteries.—The form of battery which is most commonly employed in nerve-muscle experiments is **Daniell's.** It consists

of an outer vessel of copper which forms the negative plate of the battery, full of a saturated solution of copper sulphate; in this is placed a porous earthenware vessel containing a stout rod of well-amalgamated zinc,* which forms the positive plate, immersed in dilute sulphuric acid (1 in 8). The electrical current, as soon as the two plates are connected by a wire, passes from the copper to the zinc outside the battery, and in the reverse direction within it, hence the copper is the positive pole and the zinc the negative pole. It follows, therefore, that the wire connected with the copper is the positive electrode or **anode**, and that connected with the zinc is the negative electrode or **kathode**.

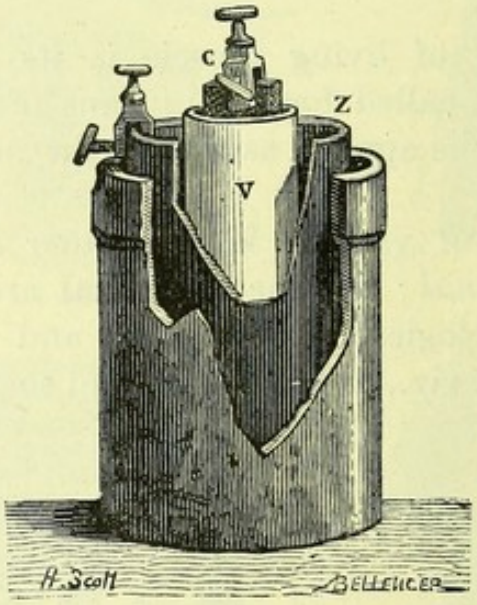

Fig. 87.—Bunsen's Battery.
C, Carbon; Z, zinc; V, porous cylinder.

The arrangement of this battery with two fluids—viz., copper sulphate solution and dilute sulphuric acid—insures an electrical current very constant in strength, which alters little during long periods, neither increasing nor diminishing. The explanation of this constancy is as follows: The dilute sulphuric acid acting upon the zinc evolves hydrogen gas; this gas, when evolved, passes through the copper sulphate solution and decomposes it into sulphuric acid [which passes inwards to renew the acid, which in the action of the battery has been converted into zinc sulphate], and copper, which is deposited upon the copper plate, and so the battery remains constant as long as the copper sulphate is replaced. This

* The rod of zinc is amalgamated, by first of all thoroughly washing it with soap—the soap used for cleansing metal—and water, then applying some strong solution of mercury in nitric acid 1 part, and hydrochloric acid 3 parts, the mercury being in excess.

is done by placing upon a ledge attached to the inside of the upper part of the copper vessel, but beneath the level of the copper sulphate solution, crystals of the salt.

In **Grove's** battery the negative element is platinum, which is contained in strong nitric acid in a central porous vessel, and the positive is zinc in the outer vessel containing dilute sulphuric acid 1 in 8. In this battery the hydrogen evolved by the action of the dilute sulphuric acid on the zinc decomposes the nitric acid with the evolution of nitrous fumes.

In **Bunsen's** battery the fluids are the same as in Grove's, but instead of platinum a negative plate of compressed carbon is substituted. This battery is chiefly used to set in motion the time-marking apparatus, to be presently described, and in cases where high electro-motive force is required. It has the disadvantage of evolving nitrous fumes.

The **bichromate** battery is one of considerable power and fair constancy. It consists of a glass vessel containing a solution of

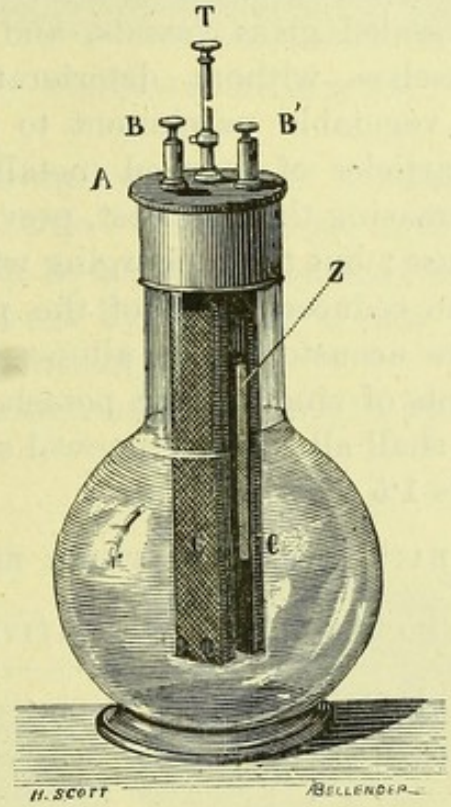

Fig. 88.—Bichromate Battery.

T, Handle for raising the zinc plate ; B and B', binding screws in connection with the plates ; Z, zinc ; A, cap, to which the carbon blocks are attached.

bichromate of potassium, to which sulphuric acid is added. The negative plate is carbon, and the positive is a zinc rod, capable of being raised out of the fluid by means of a handle attached to it.

The **Chloride of Silver Cell**, invented by De la Rue in 1868, and improved by Skrivanoff in 1883, is one of great value and of considerable scientific interest. It consists of a glass vessel containing a rod of zinc for the positive plate, and for the negative plate a silver wire surrounded by fused chloride of silver. The liquid in the modern form of cell is caustic potash. Polarization is prevented by the silver chloride, and, as this is insoluble, it remains in its proper place round the negative plate without any diffusion and consequent deposit on the zinc. In Daniell's cell the diffusion of the sulphate of copper through the porous cell is one of its chief drawbacks, for the result is a deposition of particles of metallic copper on the zinc plate, and, as a result, local action is set up, and the electro-motive force of the battery falls. This is avoided in the chloride of silver cell, which may be trusted to give an even electro-motive force from first to last until all the chloride of silver has been consumed. It is, therefore, a constant cell. Moreover, there is no consumption of zinc on open circuit, so the zinc need not be withdrawn when the cell is put away; in fact, these cells are usually sent out in sealed glass vessels, and may be left almost indefinitely to themselves without deterioration. The negative plate is wrapped in vegetable parchment to prevent any risk of short circuiting by particles of reduced metallic silver. The high price of silver, by increasing the first cost, prevents these cells from coming into general use; but the recharging with silver chloride is nearly paid for by the reduced silver of the preceding charge, for which the makers are accustomed to allow; and it is easy so to proportion the amounts of zinc, caustic potash, and silver chloride in the cell that they shall all require renewal simultaneously. The electro-motive force is 1·5 volts.

LIST OF THE CHIEF BATTERIES WITH THEIR RELATIVE STRENGTHS.

	POSITIVE CELL.		NEGATIVE CELL.		Electro-motive Force.
	Plate.	Fluid.	Plate.	Fluid.	
Daniell ...	Zinc	Dilute H_2SO_4	Copper	$CuSO_4$ satd. soln.	1·079
Grove	Do.	Do.	Platinum	HNO_3 fuming	1·956
Bunsen ...	Do.	Do.	Carbon	Do.	1·964
Bichromate	Do.	Bichromate of Potass. & dilute H_2SO_4	Carbon	As in positive cell	2·028
Leclanché.	Do.	NH_4Cl in soln.	Carbon with MnO_2	As in positive.	1·561

Wires.—The wires generally used are stout copper wires of different calibre, well insulated with gutta percha. The thinnest wires used are frequently, for the sake of flexibility, covered with fine cotton or silk.

Keys.—These are arrangements by means of which the ends of

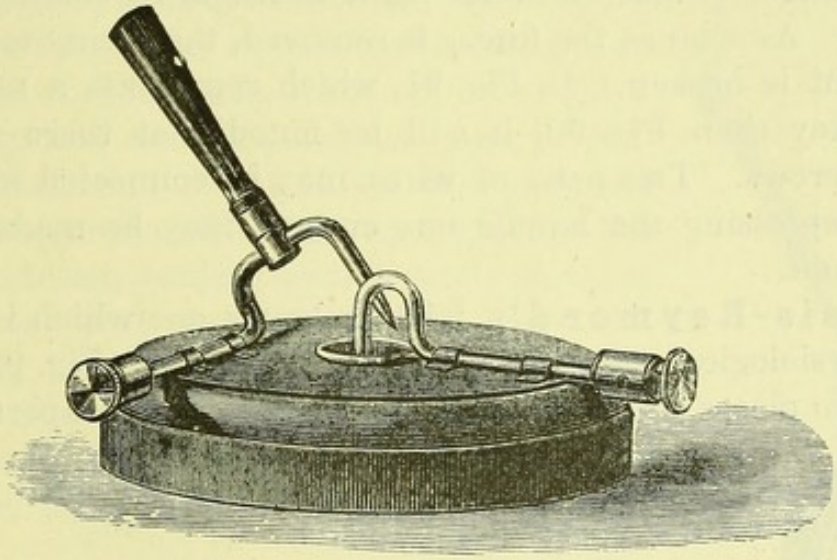

Fig. 89.—A Mercury Key.

the wires attached to the two poles of a battery may be connected or disconnected at will. Some keys have other uses, as, for example, they may be so arranged that they are capable of altering the course of a current by opening a new way for its passage. There are many varieties of key.

In Fig. 89 is shown a mercury key, in which the metallic con-

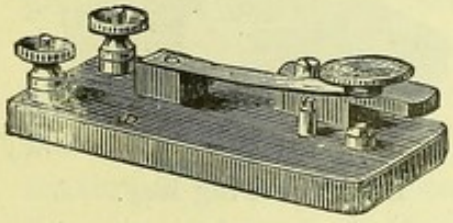

Fig. 90.—Simple Spring Key.

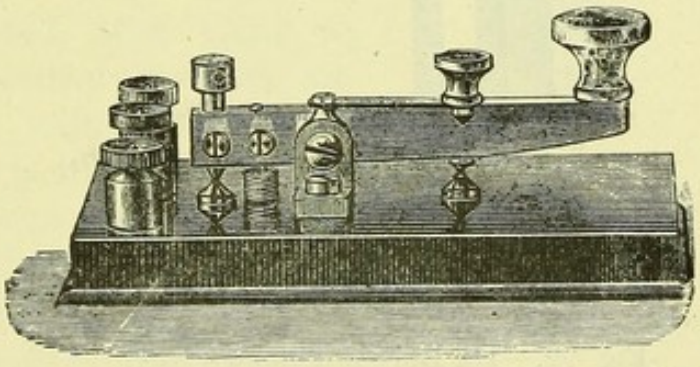

Fig. 91.—Morse's (Spring) Telegraph Key.

nection between two wires of a battery attached to the two binding screws is bridged over by depressing the handle to the left of the figure so that the wire connected with one binding screw passes into the mercury in the cup, in which the wire in connection with the

other binding screw is constantly immersed. When the handle is depressed, the current is **made**; when raised, the current is **broken**.

In Figs. 90, 91, the connection between the battery wires secured by the binding screws is made by means of a spring which is in metallic connection with one binding screw, being pressed down with the finger to touch a metal point which is in connection with the other. As soon as the finger is removed, the spring recoils, and the current is broken. In Fig. 91, which represents a more complicated key than Fig. 90, it will be noted that there are three binding screws. Two pairs of wires may be connected with them, that on depressing the handle one current may be made and the other broken.

Du Bois-Reymond's friction key is one which is of great use in physiological experiments. It will be seen (Fig. 92) to consist of two pieces of brass, each provided with two apertures and

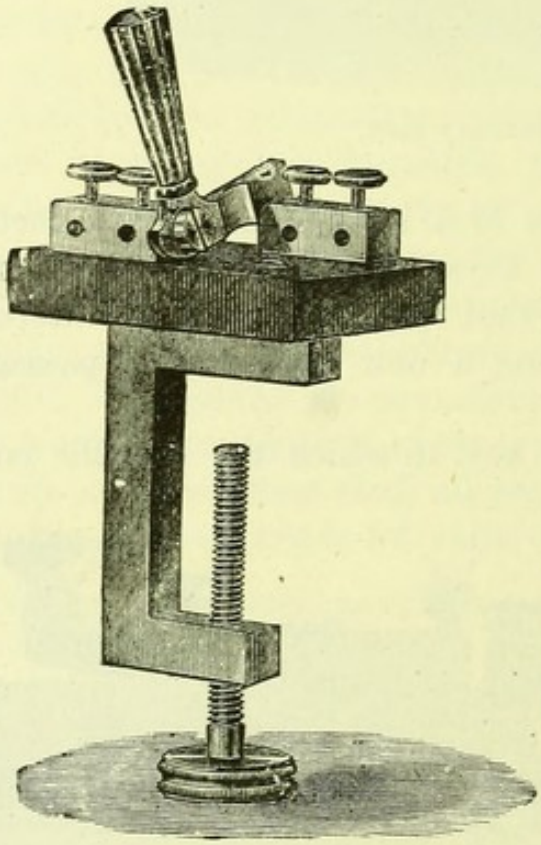

Fig. 92.—Du Bois-Reymond's Key.

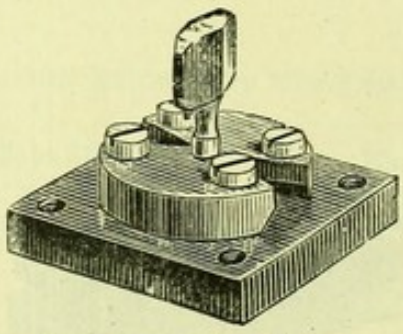

Fig. 93.—Plug Key.

binding screws for wires, fixed upon a vulcanite plate capable of being attached to the table by a screw clamp. A piece of brass fixed by a screw can be made to bridge over the interval between the fixed brass blocks by bringing the handle fixed to it at right angles to the perpendicular. When this is done, the contact between the bridge and the fixed block must be very perfect.

In Fig. 93 is shown a key, in which a connection between the fixed brass blocks is made with a brass plug.

Electrodes.—The ends of the battery wires are usually provided with special terminals, to which the name electrodes is generally restricted.

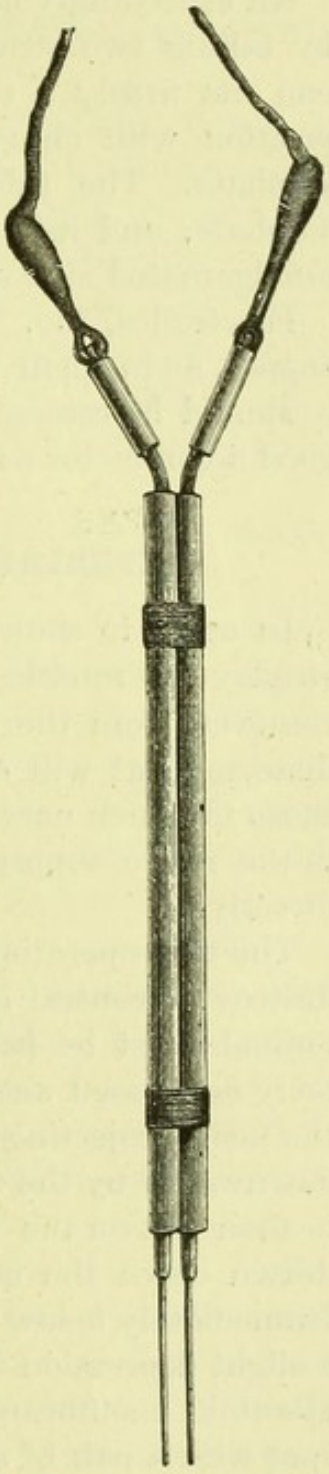

Fig. 94.—A Pair of Electrodes.

A simple pair may be made by each student by fixing two pieces of clean and bright copper wire about two and a half inches long into two pieces of glass tube about an inch and a half long, and allowing half an inch to project at either end of the tubes, filling up the tubes with powdered sealing-wax, heating the tubes, and then joining them securely together with thread and sealing-wax. To the wires projecting at one end, lengths of thin and flexible wire about three feet long, covered with cotton or silk, should be soldered, and the other ends may be bent nearly at right angles.

Convenient **cork electrodes** should also be made by inserting pins about two inches long into a cork cut into a convenient shape, soldering wires to the heads, cutting off the points, and turning the projecting parts as above.

Both these forms of electrodes are what are called **polarizable** electrodes, as in consequence of the impurity of the metal of which they are constructed, after being in use for a time, auto-electrical currents are developed in connection with them. This polarization of electrodes is likely to be very confusing in certain experiments, hence **non-polarizable electrodes** of various forms have been constructed. The principle of the formation of these electrodes is, however, always the same. A piece of glass tubing (2 to 3 inches long) is bent into the form of a U (or into some other convenient shape), and half filled with a saturated solution of zinc sulphate. The part of one limb of the U unoccupied with the solution is filled with china clay (kaolin), moistened with saline solution; a plug of the same material is pressed down upon the top of the tube nearly at a right angle, so

that it is in connection with the china clay in the tube. Into the other limb of the tube dips a well-amalgamated rod of zinc, to which is attached the battery wire.

An exceedingly useful form of non-polarizable electrodes is made by taking two straight pieces of glass, so arranged that into one end fits firmly a camel's-hair brush (without the quill), fixed in position with china clay, and moistened with a solution of zinc sulphate. The tube is filled with a saturated solution of zinc sulphate, and into it dips through an india-rubber collar a little amalgamated zinc rod, provided with a binding screw.

Electrodes, too, with **platinum** points may be used instead of copper, as in them polarization is practically done away with; but it should be remembered at the same time that platinum is not so good a conductor as copper.

EXPERIMENTS WITH THE CONSTANT CURRENT.

In order to show the excitability of living muscle, it is usual to employ the muscles of a pithed frog, either remaining *in situ* or removed from the body. Any muscle which may be conveniently dissected out will answer the purpose; but it is usual to employ those to which nerves can be easily traced, and to apply the stimuli to the nerve supplying the muscle, instead of to the muscle itself directly.

The first operation required is **to pith a frog**—*i.e.*, to completely destroy its central nervous system. In order to accomplish this the animal must be held by its two fore-legs in such a way that its belly is pressed against the dorsal surface of the left index-finger, the head projecting beyond the tip of the finger, and being pressed downwards by the thumb of the left hand. The skin on the back is then put on the stretch, and the nail of the right index-finger is drawn down the centre of the head towards the spinal column. Immediately below the head, and at its junction with the vertebræ, a slight depression will be felt, marking the position of the occipito-atlantoid membrane. A small triangular snip is made over this spot with a pair of sharp-pointed scissors; the membrane is divided, and a wooden match sharpened at one extremity is first thrust upwards into the brain to destroy sensibility, and then a long blanket-pin is pushed downwards into the spinal canal to destroy the spinal cord. In performing this operation no bleeding should occur. Care must be taken that the pin really enters the spinal canal, and that on the one hand it does not simply pass beneath the skin of the back, whilst on the other it does not pass into the

abdominal cavity. The sudden extension of the hind limbs may be taken as a proof that the destruction of the cord has been properly completed.

In order to study the effect of stimuli applied to the nerves supplying a considerable number of muscles, the following preparation should be made:

(i.) Having pithed a vigorous frog, the abdomen should be opened with a pair of sharp scissors, the viscera turned aside and removed, and the sacral plexus of each side should be exposed. The nerves should be freed from connective tissue, and the head of the frog should be clamped in a holder, and attached to a retort stand, allowing the lower extremities to hang about eight or ten inches above the table. A pair of **electrodes** should be inserted behind the nerves of the plexus.

The directions for making the more usual form of **nerve-muscle preparation**, however, are as follows:

(ii.) Pith a frog and remove the skin from the back of one thigh; this will expose the muscles enclosed in a fine sheath of fascia; with two pairs of forceps separate the muscles by tearing the sheath. Three muscles will be exposed — the *triceps*, on the outside, the *semi-membranosus* on the inside, and lying between the two, and partially covered by them, a smaller muscle with tendinous ends, the *biceps*. Follow the biceps to its origin, and carefully cut it through with a pair of scissors; then catching hold of the divided end, pull it forcibly down towards its insertion, and entirely remove it. By this method of procedure the sciatic nerve will have been exposed. Carefully dissect it out, tracing it upwards to the spinal column, and downwards to the gastrocnemius—notice its first and chief division to supply the two heads; do not prick the blood-vessels in contact with the nerve, but gently separate them. Cut through the spinal column with the spinal cord, and divide the part detached vertically into two; then by holding the piece of bone belonging to the side which is being dissected, lift up the nerve, and free it from the surrounding tissues. The nerve will now be ready for the succeeding experiments. During the operation it ought not to have been pinched, pricked, or otherwise injured.

The nerve must be placed upon the electrodes, and the preparation is ready for the demonstration of muscular

contractions under stimuli. Care must be taken lest the preparation dry up.

1. Having arranged the nerve-muscle preparation in a convenient holder, charge a Daniell's battery, and bring the wires from the copper plate to one of the brass plates of a Du Bois-Reymond's key; attach the wires of a pair of electrodes, the one to the zinc pole, and the other to the other plate of the key; have the handle of the bridge lowered, and place the electrodes behind the nerve of the preparation. On raising the handle to the perpendicular, and so closing the key, metallic connection is established, and the battery current is **made**. A contraction takes place; this is called a **making contraction**. By depressing the handle, open the key; the metallic connection is broken, and a second contraction follows. This is a **breaking contraction**.

(α) Note that between the first contraction and the second no *apparent* change is taking place in the muscle.

(β) Note also that for the purposes of the above experiment, any key might have been employed, instead of Du Bois-Reymond's, as it was only used for its simplest purpose to connect or disconnect the wires from the battery at will.

(γ) Note the direction of the current in the experiment; if the anode be placed behind the nerve above the kathode, the current passes down the nerve, and the current is said to be **descending**. If, on the other hand, the anode is below the kathode, the current passes upwards, and is said to be **ascending**.

2. Repeat the experiment, but with two additional wires, bringing the battery wires to the inner binding screws of the key, and attaching the electrode wires to the two outer binding screws. The electrodes should be placed behind the nerve as before, but before adjusting them, close the key by raising the handle. When all the arrangements are in place, open the key: this allows the current to pass into the nerve, and a making contraction takes place; close the key, practically the whole of the current is cut off from the nerve, and the battery current then passes in the short circuit across the key, and is thus what is technically known as **short circuited**, a breaking contraction at the same time taking place. The Du Bois-Reymond's key has thus been used for a second purpose, viz., to short-circuit a current; in its place a plug key or shunt (Fig. 93) might have been used, or Morse's telegraph key, by placing the battery wires to the middle and nearer binding screws (Fig. 91), and the electrode wires to the middle and further binding screws; on depressing the handle the current is made.

To demonstrate the effect of the polarization of the electrodes, prepare the apparatus to stimulate a nerve-muscle preparation, with a Daniell's battery interposing a Du Bois-Reymond's key. If the key be *opened* for some time, and then the battery be disconnected, a contraction will occur several times on closing the key, independently of any current from the battery; this phenomenon is due to **the polarization of the electrodes**: as many as twenty contractions may be shown under favourable circumstances in this way.

The apparent effect produced by a galvanic current, which is constant or continuous in its action, is not always that which has been demonstrated in the above experiments, but varies according to

(α) The strength of the current used;

(β) The direction of the current, *i.e.*, whether it is ascending or descending;

(γ) The suddenness of the current;

(δ) The irritability of the nerve-muscle preparation.

3. Take two copper wires and solder to one a piece of thin copper foil, and to the other a piece of thin zinc. Place the two plates in a beaker of saline solution, bring the wires to the inner binding screws of a closed Du Bois-Reymond's key, and connect the electrode wires with the outer binding screws. The electrodes are behind the nerves, as in the other experiments. On opening *or* on closing the key a contraction of the muscle occurs, but **not on both opening and closing it.**

The effect produced by a weak current, either ascending or descending, is best shown, however, by an arrangement by means of which the amount of a battery current used as a stimulus may be varied at will. This may be done by means of a **rheochord** of some form or other. The principle of the construction of a rheochord is comparatively simple. If the two poles of a battery be connected by a wire, as before shown, the electrical current passes from the positive to the negative pole outside the battery. The rate at which it passes depends upon various circumstances with which we have nothing to do here. If at a certain point in the circuit, as in Fig. 95, we cut the wire and introduce two wires of equal length, thickness, and composition—in fact, exactly similar in all respects—into the circuit, the current, on reaching the junction, will split, and half will pass through *a*, and half through *b*, in its passage back to the battery. If, however, instead of a wire of equal length, we substitute a wire *c*, of more than double the length of *a*, it will be found that, although the current will continue to split as before, it will no longer divide equally; but as there is more resistance to the current in the circuit *c*, a larger portion will pass along *a*,

and thus by introducing greater and greater resistance in one circuit, as, for example, not only by increasing its length, but also by diminishing the thickness of the wire used, and by employing German silver or platinum (bad conductors) instead of copper wire, the resistance in the circuit *c* becomes so great, that practically all the current passes through *a*, and this is especially the case if for the wire *a* a thick and short copper wire, *d*, is substituted. By these means it will be seen that if the battery circuit be *a*, and the electrode circuit be *b*, only half of the battery current would be used to stimulate the preparation; in like manner, by diminishing the resistance in the battery circuit, less than half of the current will be used as a stimulus, and so on.

For all delicate experiments it is usual to employ the rheochord

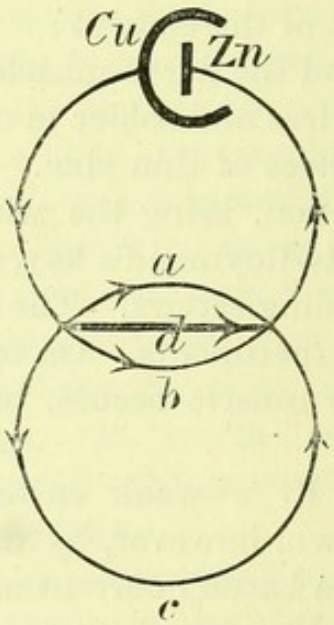

Fig. 95.—Diagram to Explain the Principle of the Rheochord.

of Du Bois-Reymond, the resistances in which have been carefully regulated. It is, however, quite easy for the student to construct for himself a simple apparatus.

Du Bois-Reymond's rheochord consists of a long box or board, on which the resistance wires are stretched. At one end are fixed several brass blocks, separated from one another, and disconnected except by fine German silver wire. At each corner of this end are binding screws, each connected with the nearest brass block. Beginning at the block most to the right, a wire passes a considerable distance up the board, passes round a peg and returns to the second block, from which a wire, in a similar manner, considerably shorter, connects it with the next block, and so on. From the two blocks at the end of the rod two thin platinum wires

pass to the opposite end of the board, and are there insulated; they are, however, connected by means of a slide, formed of cups of mercury. This slide is capable of being moved up and down the wires. The brass blocks are, moreover, capable of direct connection (except the two at the corner of the board, between which are the travelling mercury cups) by the insertion of plugs. Supposing the rheochord is interposed in a battery current in place of a key, and to the binding screws the wires of the battery are attached, as well as the electrode wires: if the slide be close to the brass blocks and all the plugs are in place, the rheochord, offering no resistance to the current, allows it to return to the battery, and none of it passes into the nerve. If the slide be pushed a short distance down the wires, the current meets with a certain amount of resistance, and so a fraction of the whole battery current will pass into the nerve. In like manner a greater and greater current passes into the nerve, if the slide be pushed farther away from the blocks, and if the plugs be removed one by one, as in this way the resistance offered to the passage of the battery current through the rheochord is more and more increased.

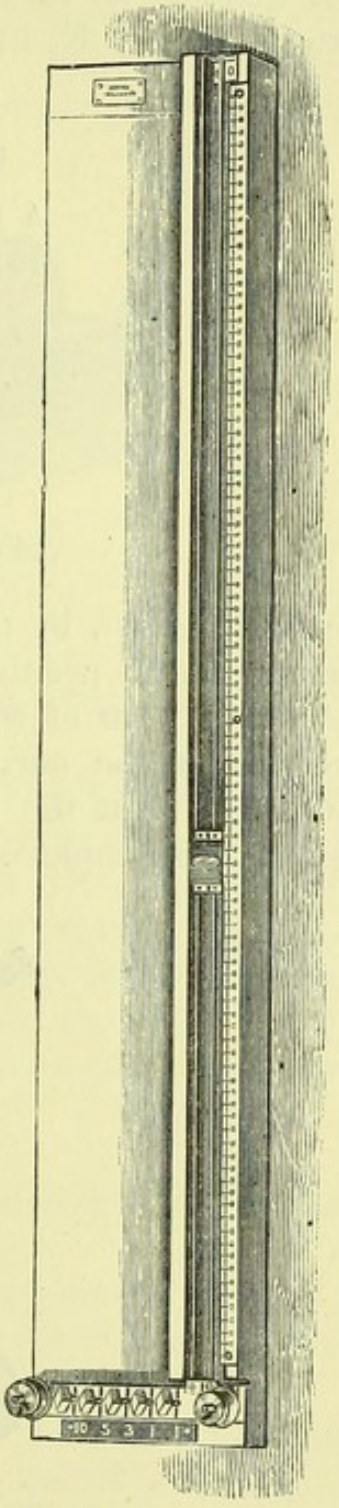

Fig. 96.—Rheochord of Du Bois-Reymond.

In order to show the effects of altering the direction of the current with currents of different strength, it is necessary to employ some kind of **reverser** or **commutator**.

In Fig. 97 is seen the form in common use. The six cups in the vulcanite plate are filled with mercury, and the wires from the battery or rheochord are attached to the two binding screws in connection with the square carrying bridge, which is not in metallic connection, but is formed by two pieces of copper wire joined together by a non-conductor—*e.g.*, vulcanite, and the electrode wires are joined either to the right or to the left of the figure. The mercury cups on the right and left are connected together by the wires seen

in the figure. If in the figure the anode be connected with the binding screw A, the positive electrode will be A, and that connected with A′ and likewise K′ to K will be the course of the current from the negative electrode. But if the bridge is turned over to the right so that there is no metallic communication between A and A′, and between K′ and K, and the wires dip into the cups at the right of

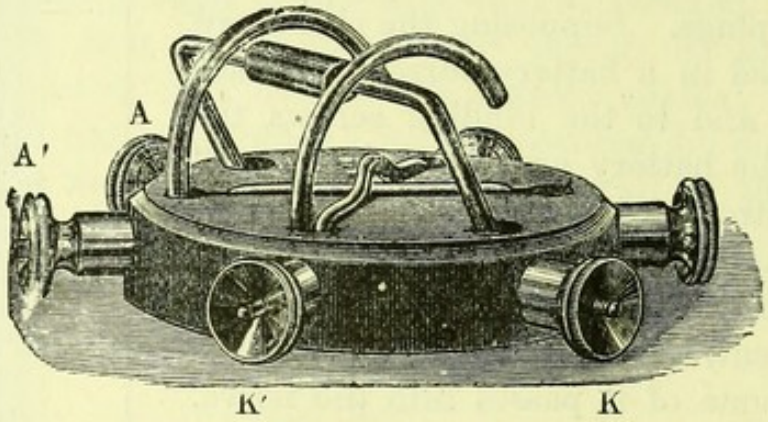

Fig. 97.—Pohl's Commutator.

the figure, then, by means of the crossed wires, K′ becomes connected with the positive electrode, and A′ with the negative.

Another form of commutator is seen in Fig. 98, in which the direction of the current is changed by raising or lowering the handle. When the wires are removed from the reverser, as in Fig. 99, it is employed for sending a current in one or other direc-

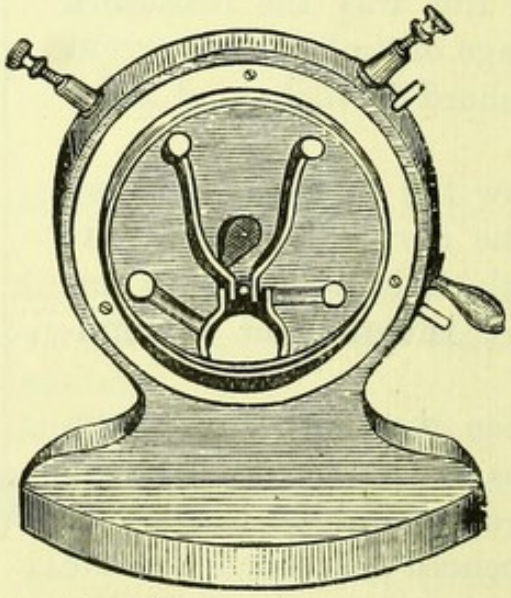

Fig. 98.—Thomson's Commutator.

tion, the binding screws to the right and left of the figure being in connection with different sets of electrodes.

4. Charge a Daniell's battery and connect the poles with the end binding screws of a Du Bois-Reymond's rheochord; connect the same

screws (the apparatus is fitted with double binding screws) with the central binding of a Pohl's commutator and the electrode wires connected with the side-screws, but connect them with a Du Bois-Reymond's key arranged for short circuiting. Place the electrodes behind the nerve of a nerve-muscle preparation. Arrange the apparatus so that the current is **descending**. First of all see that all the plugs are firmly in the rheochord, and that the travelling

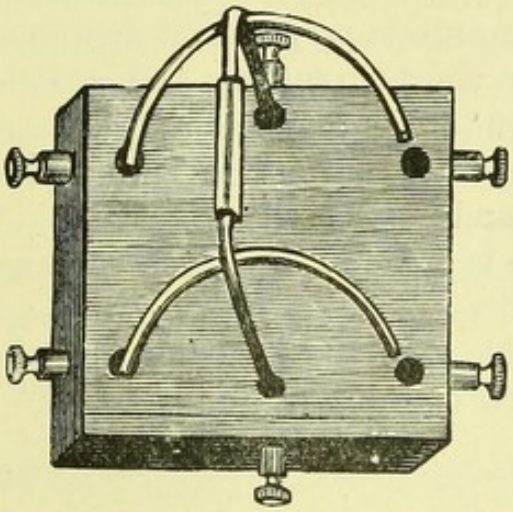

Fig. 99.—Reverser, with Connecting Wires removed.

mercury cups are pushed well home; the rheochord in such a case acts as a key, and there being practically no resistance, all the current passes through the rheochord from the positive to the negative pole, and, in fact, the battery current is short-circuited. Open and shut the key, no contraction will occur either at opening or at closing.

Next gradually push the travelling mercury cups along by the platinum wire until a point is reached where a contraction occurs. Note:

(*a*) That it is a **make** contraction; and

(*b*) Note also the distance in millimeters that the mercury cups are away from the zero of the scale.

(*c*) Proceed in the same manner until a point is reached at which a contraction occurs, both at the make and also at the break of the current.

In order to be perfectly certain of the accuracy of the experiments with the rheochord, non-polarizable electrodes should be used.

5. Repeat the experiment, but use an **ascending** current instead of a descending one. Note:

(*a*) That the contraction which first appears is a **making contraction.**

(*b*) That it occurs when the mercury cups are further away than with a descending current; and

(*c*) That the second contraction also does not appear until the current is stronger than in the case of the descending current.

These experiments (4 and 5) show clearly both that the effects of the galvanic current vary with the strength of the current, and also with the direction of the current, the descending current being with weak currents much the more powerful.

6. Obtain a medical battery with a large number of cells in order to stimulate a nerve-muscle preparation, arranging it with a Du Bois-Reymond's key for short circuiting, and a Pohl's commutator for reversing the current. Note:

(*a*) That there is a contraction with a descending current on making and not on breaking.

(*b*) That there is a contraction with the ascending current only on breaking.

In this way a table may be constructed which is not infrequently called Pflüger's **Law of Contractions.**

STRENGTH OF CURRENT.	DESCENDING CURRENT.		ASCENDING CURRENT.	
	Make.	*Break.*	*Make.*	*Break.*
Very weak	Yes.	No.	No.	No.
Weak	Yes.	No.	Yes.*	No.
Moderate	Yes.	Yes.	Yes.	Yes.
Strong	Yes.	No.	No.	Yes.

7. Arrange a nerve-muscle preparation and the apparatus for stimulation with a constant current; use a weak descending current; note that when the key is closed sharply a contraction occurs, but if it be closed gradually the contraction may not take place. The **suddenness** of the shock influences the effect of the galvanic stimulus.

8. Arrange three or four Daniell's batteries to stimulate a nerve-muscle preparation, using a Du Bois-Reymond's key, a Pohl's commutator, and non-polarizable electrodes. Allow an ascending current to pass into the preparation for several minutes, then shut it off by short circuiting; the muscle shows, instead of a single contraction, a series of twitchings, or even a complete tonic contraction; this

* It should be mentioned that not infrequently the break ascending contraction appears before the make ascending contraction.

abnormal result is called **Ritter's tetanus**, or Ritter's ascending break tetanus; it may be stopped by passing a current in the same direction into the nerve.

The Ritter's tetanus may best be demonstrated upon a frog's muscles in the breeding season. It appears to be due to some condition at the anode, as it ceases if the nerve is divided below the anode, but not if it be divided between the electrodes.

9. Under abnormal conditions, as, for example, when the frog has been poisoned with strychnine, a single stimulus will produce a prolonged tetanus. Thus the effect of the galvanic current may be said **to vary with the irritability of the nerve-muscle preparation.**

The above experiments demonstrate the effects of a constant or continuous current upon a nerve-muscle preparation which are apparent; other effects which are not apparent will be treated of later on.

EXPERIMENTS WITH THE INDUCED CURRENT.

In physiological experiments it is more usual to employ the induced or faradic than the galvanic current.

The induced current is most commonly obtained by the use of the induction coil (Fig. 100), or inductorium of Du Bois-Reymond.

This apparatus consists of two coils of wire, one called the **primary coil**, *c*, which is made up of a number of coils of thick copper wire, thoroughly insulated, wound round a wooden reel, the interior of which is packed with iron wire; this is fixed to the wooden upright, and the ends of the coil of wire are attached to the binding screws at the upper border; the other, or **secondary coil**, *g*, is made up of a much larger number of coils of fine insulated copper wire, the ends of which are connected with two binding screws, only one of which is represented to the extreme left of the figure, the wire being wound round a wooden spool, which is capable of being moved along a grooved and graduated scale nearer to or farther away from the primary coil. It is so arranged, too, that it can pass over the latter close up to the wooden upright. At the front of the apparatus, as seen in Fig. 100, is an arrangement, *a*, *b*, *d*, *f*, called the **Neef-Wagner hammer** (p. 294), for automatically making and breaking the primary circuit with rapidity. An induced current is set up in the secondary coil, at the instant when a current from a galvanic battery is made to pass through the primary coil, and a second induced current is set up, when a galvanic current, which has been passing through the primary coil, is shut

off from it. These induced currents are called respectively **make** and **break currents.**

The strength of the induced current not only depends upon the strength of the battery or batteries employed, the number of coils of wire in the primary and secondary coils, and the thickness of the wire used, but also upon (1) the *distance* the secondary coil is from the primary (the further off the weaker); (2) the *position* of the secondary to the primary—if at right angles, no induced current is obtained; (3) whether a make or a break induction—the latter is always the stronger.

1. Connect the wires of a Daniell's battery with the two binding screws, *d*, at the upper part of the wooden upright of the induction-

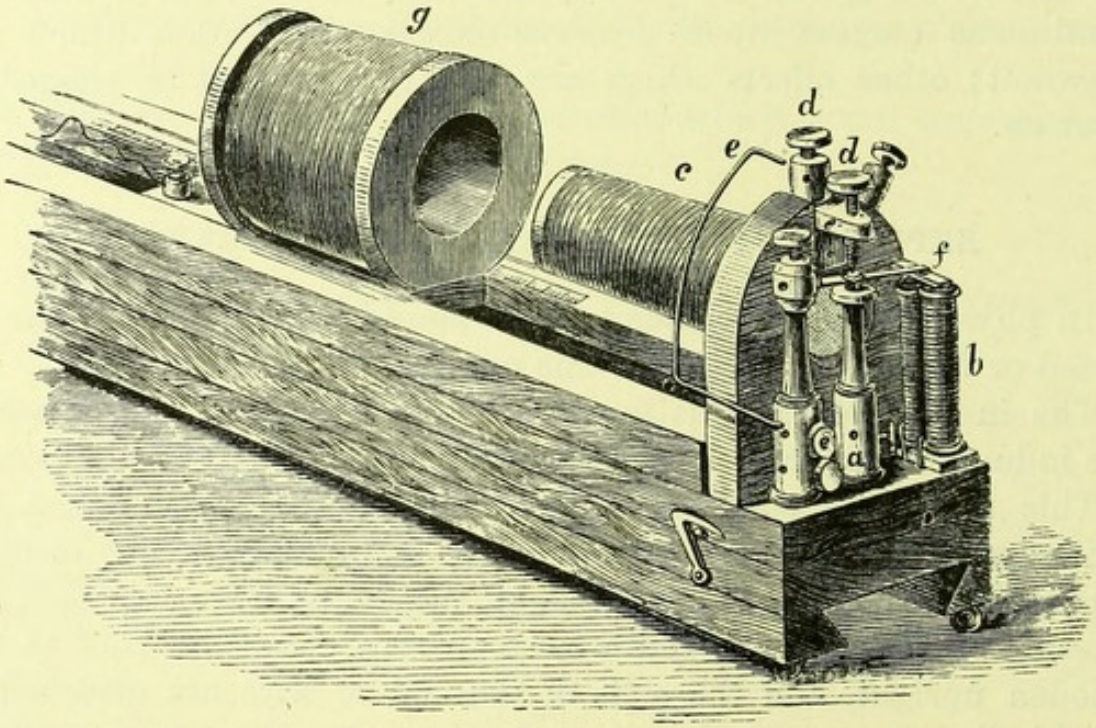

Fig. 100.—Induction Coil of Du Bois-Reymond.

coil, interposing a key of some form: a mercury key or a spring key will do equally well; connect the wires of a pair of electrodes with the binding screw of the secondary circuit, place the points of the electrodes upon the tongue, bring the secondary coil over the primary; on opening and on shutting the key the tongue will experience a sharp shock. Now move the secondary coil 15 cm. from the primary, and repeat the experiment, the shocks will be found to be much weaker; repeat the experiment with the secondary coil at 30 cm. from the primary, little if any shock will be felt, proving that the distance of the secondary coil from the primary coil is of considerable influence in regulating the strength of the induced currents.

2. Repeat these experiments, but use a nerve-muscle preparation.

3. Arrange the apparatus as in (1), but remove the secondary coil from the slide, retaining the electrode wires, the electrodes being placed behind the nerve of a nerve-muscle preparation; place the secondary coil in a line with the primary at, say, a distance of 25 cm., and note the strength of the contraction on breaking the primary circuit; then gradually rotate the secondary coil, and note the gradual falling off of the strength of the stimuli until when the coils are at right angles no contraction takes place.

4. Slide the secondary coil a considerable distance from the primary coil, open and shut mercury key—no contraction occurs. Move secondary coil nearer primary coil until a contraction ensues; note that contraction first appears when key is opened (*breaking contraction*): note the distance in millimeters; proceed until a contraction appears also on closing key (*making contraction*). *The breaking contraction always appears before the making.*

5. In a preparation in which the sciatic nerve is dissected out, but not cut, proceeding in an exactly similar way to (1), note results (in form of a table) with platinum electrodes placed behind nerve, (*a*) high up, (*b*) mid-way, (*c*) close to muscle, and also (*d*) with electrodes upon gastrocnemius itself. *The stimulus is increased as it descends the nerve.*

6. Divide the nerve high up, and repeat experiments; compare results. *When the nerve is divided, its irritability is at first increased in the neighbourhood of the section* (**Ritter-Valli law**).

7. In a preparation which has been kept cold in ice; and

8. In another, which has been warmed moderately, repeat the above experiments. *Temperature affects irritability.*

9. If time permit, repeat experiment (2) with the same preparation after an hour has elapsed, and again after a second hour, and compare results. *Irritability gradually diminishes some time after section of the nerve has taken place.*

Always be careful that your battery is working, your wires unbroken, your contacts secure, and your metallic connections bright.

The use of the key in the secondary circuit is always advisable, as by it it is possible to cut off the make or break contraction, or both; thus if key 2 is open whilst key 1 is opened and shut, contractions will occur at make and break. If key 2 is open, and then key 1 is opened, a breaking contraction will occur; but the making contraction may be cut off by closing key 2 before key 1. And similarly, if key 2 is closed and then key 1 is opened, and opened before key 1 is shut, a single making contraction will occur. If key 2 is closed whilst key 1 is opened and shut, no contraction will occur.

10. Connect the wires from the battery with the screws at the bases of the two pillars of an induction coil (*c*, *a*). Interpose a key in the secondary circuit. The current passes up the outer pillar, along the spring, until it arrives at the point where contact is made by means of a platinum-pointed screw (*e'*), adjustable, and in connection with the end of the primary coil; the current passes by this connection through the primary coil, and then through the coils of wire surrounding two pieces of soft iron, and thence to the battery through the middle pillar coil. As soon as the current passes through the electro-magnet, the soft iron is magnetized, and draws down the hammer. This breaks contact with the spring, and the current is by this means interrupted, to be again made as soon as

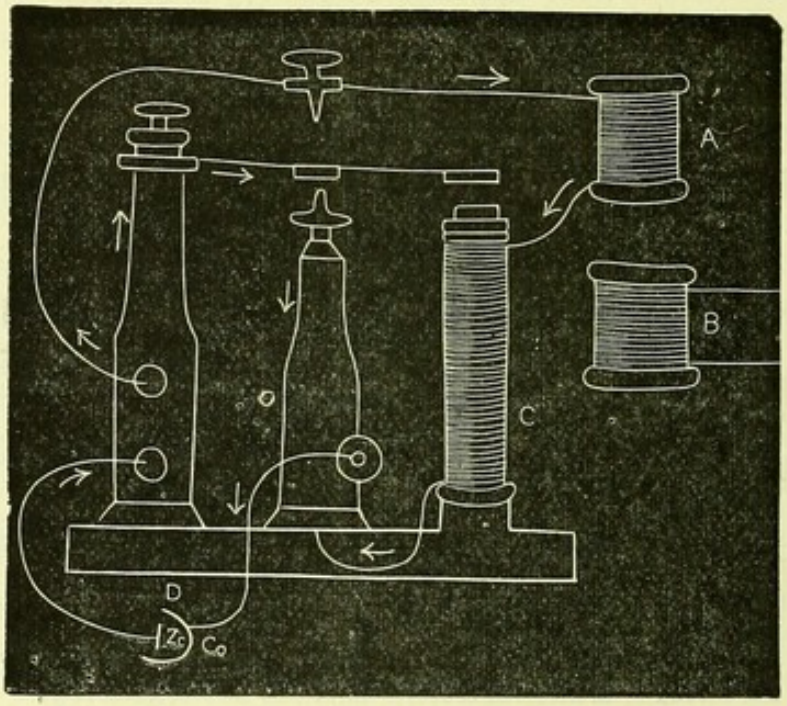

Fig. 101.—Diagram of the Course of the Current through the Induction Coil when Helmholtz's Connecting Wire is used.

the magnet ceases to act from the stoppage of the current through the electric coil round its soft iron. In this way a series of rapid make and break shocks occur, and these are represented in the induced current, and therefore in a series of make and break contractions of the muscle, when the electrodes placed under the nerve are connected with the screws of the secondary coil, and the key is opened; the effect of this is to send the muscle into a state of tonic spasm, or **tetanus**. The frog's leg and foot are rigidly extended. The contraction will continue for some time, but will finally give way under prolonged stimulation.

The apparatus is known as the **Neef-Wagner hammer** or **magnetic interrupter**. With the magnetic interrupter thus em-

ployed, the break current is found to be much more powerful than the make, and so the stimuli are not uniform in strength as they should be to produce a true tetanus. A modification, therefore, is necessary, the object of which is to equalize, as far as possible, the make and break shocks. This consists of a stout copper wire which connects the outside pillar of the apparatus directly with a binding screw in connection with the screw at one end of the primary coil; in this way part of the current always passes at once to the primary coil, and only a portion of it is made and broken by the interrupter, whether or not the plate is attracted to the magnet. The point of the middle pillar must be raised by means of the middle screw, and the screw attached to the end of the primary coil must also be screwed up away from contact with the spring. The current on entering (supposing the contact between the spring and the middle pillar to be made) divides; one portion passes through the primary circuit and magnet, the other passes through the contact down the middle pillar and back to the battery (Fig. 101); but as the part of the current which passes through the magnet is insufficient for the purpose of retaining the hammer in contact, the current is broken by the hammer springing away from the magnet; then the whole of the battery current passes through the primary coil and magnet, and again the hammer is attracted to the latter, and so on. It will be seen that by this arrangement only a portion of the current is interrupted.

11. In order to understand the cause of the difference in the strength of the making and breaking inductions, the **break extra current of Faraday** may be demonstrated by taking a Daniell's element, two Du Bois-Reymond's keys, the primary coil, a nerve-muscle preparation, and electrodes. The apparatus (Fig. 102) is arranged so that both keys, as well as the induction coil, are placed in the primary circuit, whilst the electrodes are connected to the second key. On testing the current with the tongue, supposing the key C to be open, on opening the second key, but chiefly on shutting it, there is an appreciable effect upon the tongue. When the coil is cut off by closing key C, there is very little or no effect on opening the second key. The effect is produced by the extra induced current. It may also be shown by diminishing the battery current by a wire directly connecting the poles until the galvanic current passing to the nerve-muscle preparation is too weak to cause a contraction. On breaking the primary coil a contraction will occur.*

* Authorities are by no means agreed as to the exact explanation of the greater potency of the break stimulus over the make. Foster (Ed. v., p. 64)

12. The make and break induction shocks may be equalized **by derivation**, by connecting the two ends of the primary coil by a length of stout copper wire. Arrange the apparatus for single induction shocks, but connect the binding screws of the primary coil,

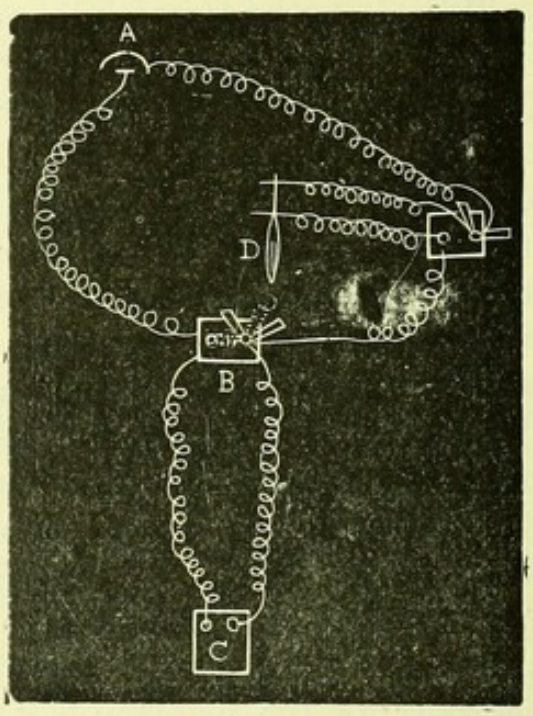

Fig. 102.—Diagram to show the Demonstration of Faraday's Extra Current.

A, The battery; B, Key No. 1; C, Primary coil; D, Nerve-muscle preparation.

interposing a mercury key. As long as this key is kept closed the make and the break inductions produced by shutting the key in the primary circuit are more equal, but much weakened in strength.

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gives the following: 'The current from the battery, upon its first entrance into the primary coil, as it passes along each twist of that coil, gives rise in the neighbouring twists of the coil to a momentary induced current having a direction opposite to its own, and therefore tending to weaken itself. It is not until this self-induction has passed off that the current in the primary coil is established in its full strength. Owing to this delay in the full establishment of the current in the primary coil, the induced current in the secondary coil is developed more slowly than it would be were no such "self-induction" present. On the other hand, when the current from the battery is *broken* or *shut* off from the primary coil, no such delay is offered to its disappearance, and consequently the induced current in the secondary coil is developed with unimpeded rapidity. . . . A rapidly-developed current is more effective as a stimulus than a more slowly-developed current.' And again, as regards Helmholtz's modification, the same author says (p. 68): 'Since, at what corresponds to the *break* the current in the primary coil is diminished only, not absolutely done away with, self-induction makes its appearance at the *break* as well as at the *make;* thus the *breaking* and *making* induced currents or shocks in the secondary coil are equalized. They are both reduced to the lower efficiency of the *making* shock in the old arrangement.'

13. **Unipolar Excitation.**—Arrange the battery and coil for single induction shocks, and connect one electrode with one of the screws at the end of the secondary coil. Place this under the exposed sciatic nerve of a nerve-muscle preparation, which should be arranged on a plate of glass upon a frog board—*i.e.*, a flat piece of board covered with cork. Open and shut a key in the primary circuit, and there will be no response; now touch the muscle with the finger or a pair of forceps held in the hand, and it will contract.

14. **Galvani's Experiment.**—Take a piece of zinc, thoroughly cleaned, and coil round one end a piece of copper wire,

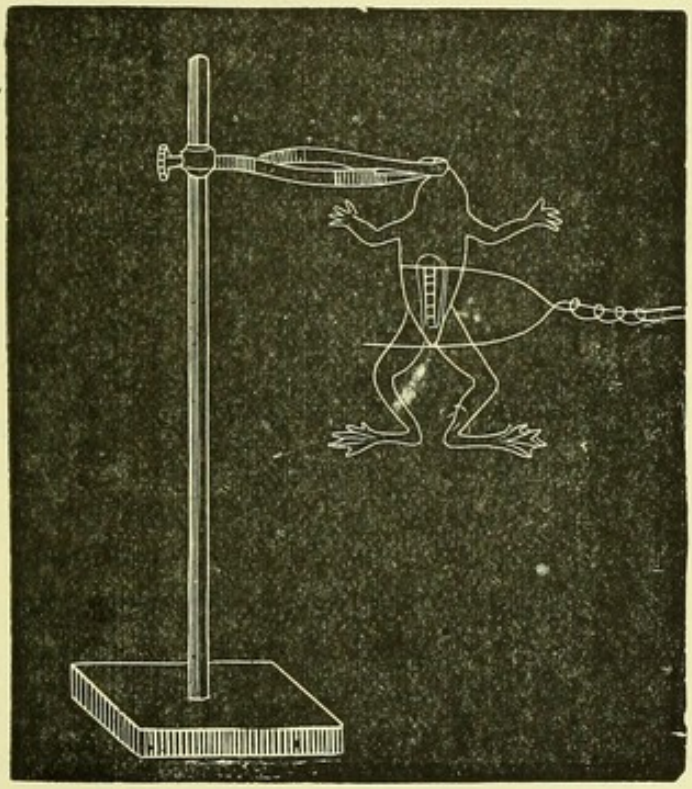

Fig. 103.—Galvani's Experiment.

which projects in such a way that a fork with two equal prongs is made. Insert the zinc behind the lumbar nerves of a pithed frog, and allow the copper to fall upon the thigh muscles; a contraction will occur. If the muscles be very excitable there will also be a contraction when the copper is removed from the muscle.

15. **Interruption by means of a Spring.**—Bring one of the wires from a Daniell's cell to the end of a steel spring (Fig. 104) which is fixed in a support in such a way that when it is set into vibration the opposite end dips into a mercury cup in metallic connection with the primary coil, and which is also connected with the battery (the spring and cup taking the place of a key); connect the

electrodes with the secondary coil, and place them under the sciatic nerve of a pithed frog. Make the spring vibrate, and notice that if the spring is short, tetanus is at once set up, and if long, that one contraction is distinctly piled on the top of another at first, and that a more gradual tetanus is produced.

16. **Interruption by means of a Metronome.**—Insert a vibrating metronome into the primary circuit of an induction coil, and allow the series of induction shocks thus regulated to break into a nerve-muscle preparation. The limb will gradually pass into tetanus.

Simple Tetanus Spring or Vibrating Contact Maker.—This was first designed for studying the tetanus of muscles, but is useful for any experiment requiring for a short time the rapid periodic interruption of an electric current. A long straight spring is gripped in a brass block, which is clamped to a

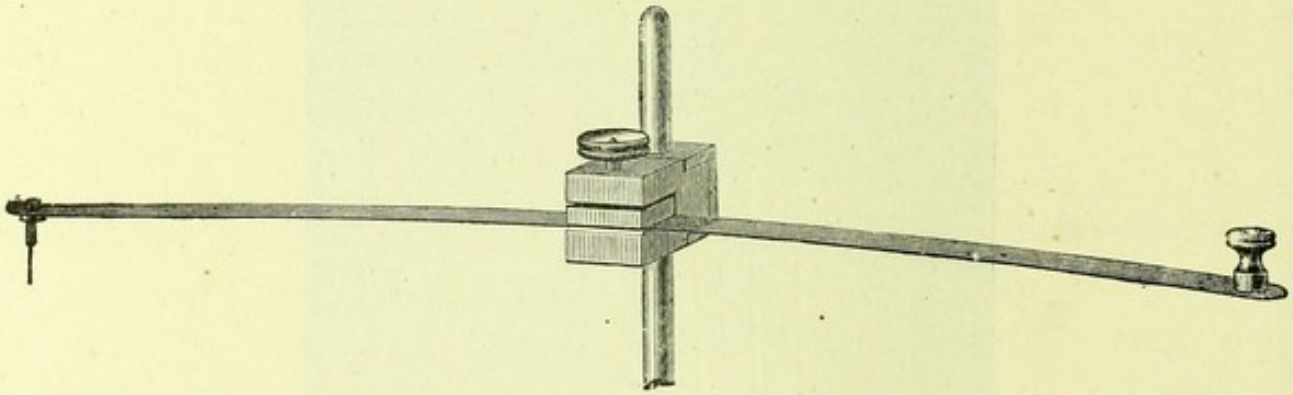

Fig. 104.—Vibrating Spring.

simple stand in such a manner as to allow of its free vibration. At each vibration of the spring a platinum wire, fixed to its end, dips into a mercury cup, and completes an electric contact. One terminal is connected with the cup, and the other with the spring. The instrument is set in action by deflecting the spring with the finger and then freeing it, when it will vibrate for a short time, giving an intermittent current. The speed of vibration can be varied within wide limits by gripping the spring at different distances from its end.

EFFECTS OF URARI.

Destroy the brain of a frog, and then dissect out the right sciatic nerve of one leg, disturbing the parts as little as possible. Pass a ligature under the nerve and tie it tightly round the thigh, excluding the nerve. Next inject with a capillary pipette 1 to 3 drops of a 1 per cent. aqueous solution of urari into the posterior lymph-sac of the frog. Set the preparation aside under a bell-glass, taking care

to keep it moist. At the end of an hour dissect out the sciatic nerve of the left leg, remove the skin from both legs, and perform the following experiments:

(*a*) Stimulate the left sciatic nerve with a tetanizing induction current; the gastrocnemius does not contract.

(*b*) Stimulate the left gastrocnemius directly; it contracts.

(*c*) Stimulate the right sciatic nerve; the right gastrocnemius contracts.

(*d*) Stimulate the right gastrocnemius directly; it contracts.

The above experiments prove that the poison does not act upon the muscle itself, or upon the nerve itself, but upon something which is intermediate, and it is taken to mean, therefore, that urari paralyzes the nerve-endings in muscle, *i.e.*, the motorial end plates.

(*e*) Remove a muscle from a well-urarized frog, and place it upon wire electrodes some little distance apart, connect these with a Daniell's battery, fatigue the muscles by rapidly opening and shutting the key, then notice that at last only the part of the muscle near the kathode contracts on make, and that near the anode on break.

OTHER FORMS OF STIMULI.

1. Prepare a nerve-muscle preparation; pinch or prick the nerve; a contraction will occur. This is an example of **mechanical** stimulation.

2. Touch the nerve or muscle of a nerve-muscle preparation with a hot needle; a contraction will result. This is an example of **thermal** stimulation.

3. Allow the nerve of the same or of a new nerve-muscle preparation to dip into a watch-glass full of strong saline solution; flickering contractions which may pass into tetanus will result. A similar experiment may be performed with glycerine. Ammonia will stimulate muscle, but not nerve; glycerine has the reverse effect. This is an example of **chemical** stimulation.

THE GRAPHIC METHOD.

This consists in arranging the nerve-muscle preparation in such a way that, on contracting, the muscle acts upon a lever, which lever is made to mark on a moving surface. The muscle and nerve may be removed from the body, or may be retained *in situ*. The recording apparatus may be a revolving cylinder covered with blackened paper, a pendulum myograph, or a spring myograph; of all of these apparatus there are many varieties.

The Recording Cylinder (Fig. 105).—This apparatus usually consists of a cylinder or drum, which is arranged to move upon axes, revolving at definite and different rates, by means of a

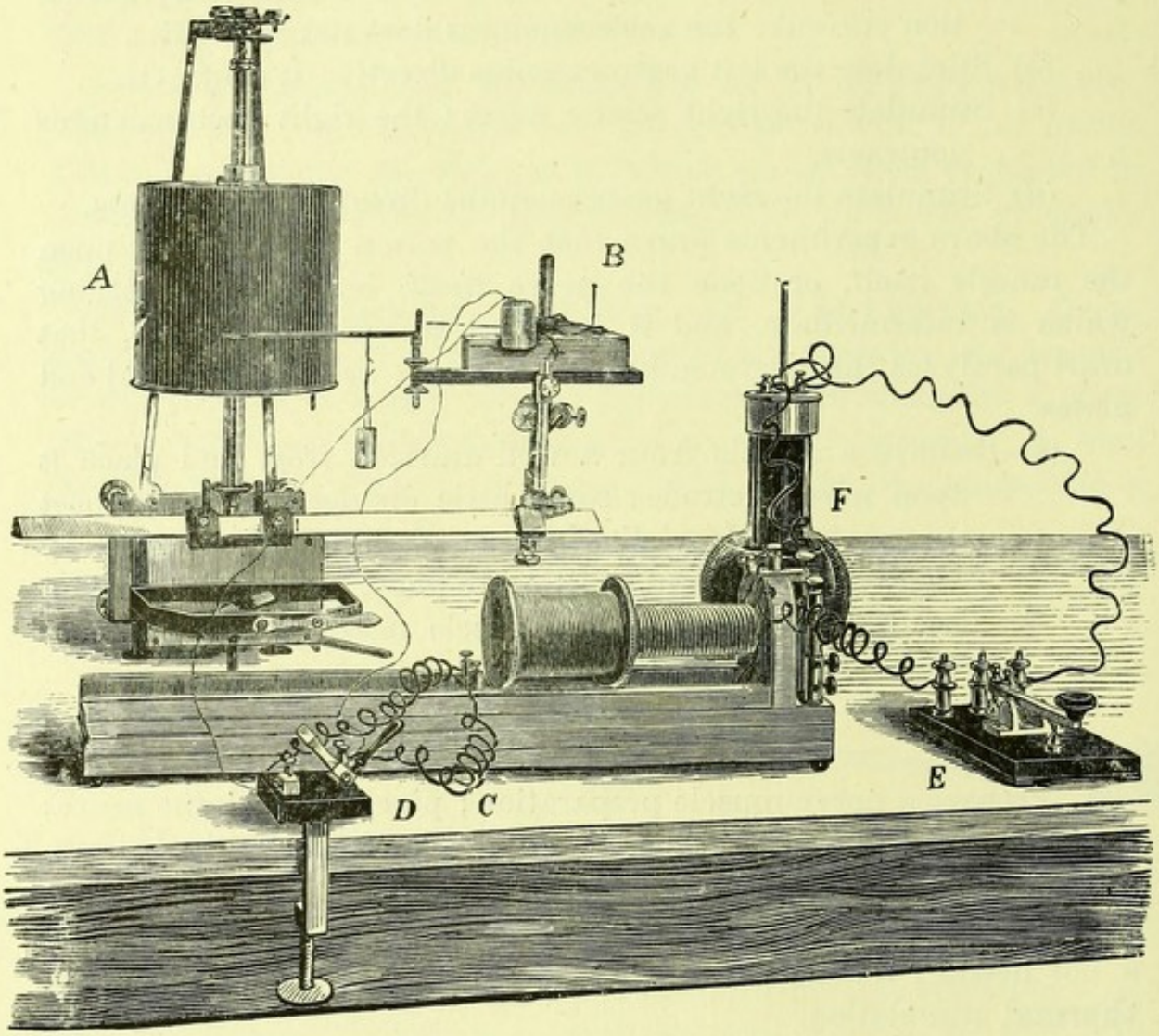

Fig. 105.—Arrangement of Apparatus for recording with a revolving cylinder the Contractions of Muscle.

A, The cylinder or drum which is situated on the most anterior and most rapidly-revolving axis. It is in connection with the brass box containing the clockwork. Two other axes are seen behind, and on either side of that bearing the drum. B, Frog on a support; its muscle is connected by a thread with the myograph: the whole being supported upon an upright springing from a triangular steel rod. C, Du Bois Reymond's induction coil. D, Du Bois Reymond's key. E, Morse's key in primary circuit. F, Bichromate battery.

clockwork mechanism contained in a brass box, which rests firmly upon supports. The velocities of the movement are:

(1) Slow;
(2) Medium;
(3) Fast.

The axes of 1 and 3 move in the same direction, viz., from right to

left; 2, on the other hand, moves in the reverse direction. By means of a screw in the axis of the drum itself the drum may be raised or lowered at the will of the operator. The apparatus works as well when placed in a horizontal as in a vertical position. In the front of the box is arranged a fan in a metal frame, which regulates the clockwork movement. The movement may be stopped by means of a metal clip and handle, which are fixed so that when the handle is pressed down the clip catches a steel axis about which the fan revolves, and the clockwork is stopped. Underneath the case of the clockwork is a handle, by which it is wound up. For the sake of convenience, in the front of the brass box of the clockwork are two screws, by which is fixed a metal frame, carrying a long,

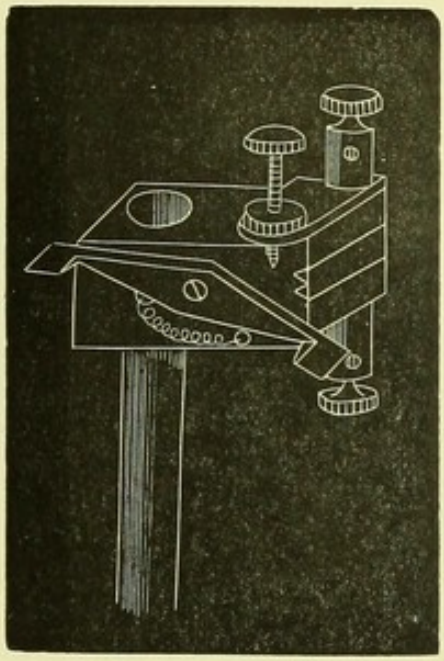

Fig. 106.—Trigger or Kick-over Key.

stout, triangular steel bar, which can be adjusted at different positions and lengths, by means of screws in the frame. On this triangular bar various metal uprights for carrying apparatus can be arranged. Also at one point of the lower edge of the drum is attached a metal catch for the purpose of opening a key placed in its way, called a **kick-over** or **trigger key** (Fig. 106), the use of which is to have the muscular contraction recorded exactly at the same place on the cylinder.

Having mastered the mechanism of the apparatus, the student should then arrange it for work. The drum should be evenly covered with glazed paper, which is generally kept ready cut in strips of the size of the drum. A strip should be placed round the drum, care being taken that the junction should not be near the metal catch mentioned

above, as in that case the tracing of the lever will be over the join, and so be spoiled. Having firmly and evenly covered the drum, it must be blackened over the smoky flame of a small paraffin lamp or a spirit lamp, in the spirit of which camphor has been dissolved For these operations the drum will have been removed from its axis; it may now be replaced, the clock wound up, the metal bar firmly fixed, and everything arranged in readiness for the recording operation.

Method.—As above mentioned, the muscle and nerve may remain *in situ* or be removed from the body. First of all use the apparatus already described. This consists of a triangular piece of wood, covered to a certain extent with cork, and with an upright cylinder of the same material fixed at the side. In front is a lever arrangement, by which the movement of the muscle during contraction is communicated to the recording apparatus. On one side is fastened a small collar, which can slide up or down an upright fixed to the

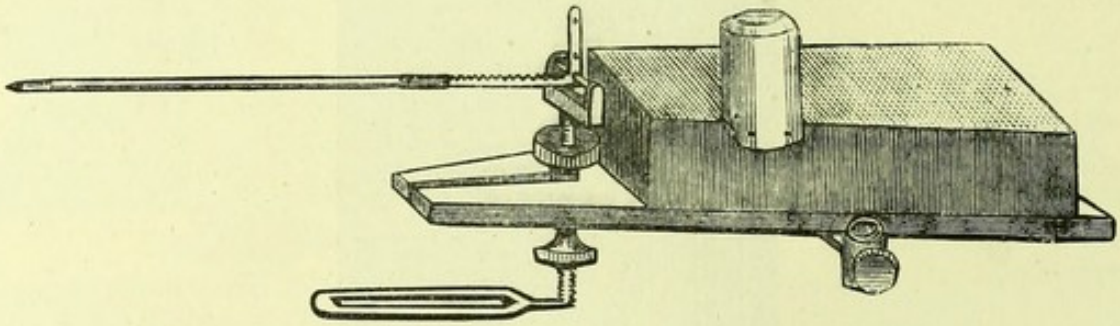

Fig. 107.—Sanderson's Myograph.

triangular rod above described, and by means of a screw can be secured at any height which may be required.

In a nerve-muscle preparation, such as ii. (p. 283), remove the skin from the foreleg of the frog, and having cut through the tendon of the gastrocnemius at the *os calcis*, turn it up and cut off the remainder of the leg just below the knee-joint. Attach a strong ligature of silk or thread to the tendon, place the frog on the cork plate, firmly fix the limb by passing a long pin through the knee-joint, and then attach the ligature from the tendon to the metal at right angles to the marking lever, carefully noticing that the ligature is taut, and that the muscle is really pulling on the lever; load the lever with a 10 or 20 grm. weight, and fix the myograph on the upright before spoken of, which slides along the triangular steel bar, so that the lever touches lightly the blackened surface of the recording cylinder, with the point writing the proper way. The apparatus is now arranged.

Moist Chamber. — This consists of a movable platform, sliding up and down the upright of a stand not unlike a medium-sized retort stand, and capable of being fixed by means of a collar and screw. The platform is made of hard wood or of vulcanite, about $3\frac{1}{2} \times 2\frac{1}{2}$ inches, and is furnished with two sets of binding screws for electrical purposes. These screws are continuous with wires which pass through the platform and project below, so that battery or other wires may be attached to them. Inside the corresponding binding screws are connected with two pairs of electrodes.

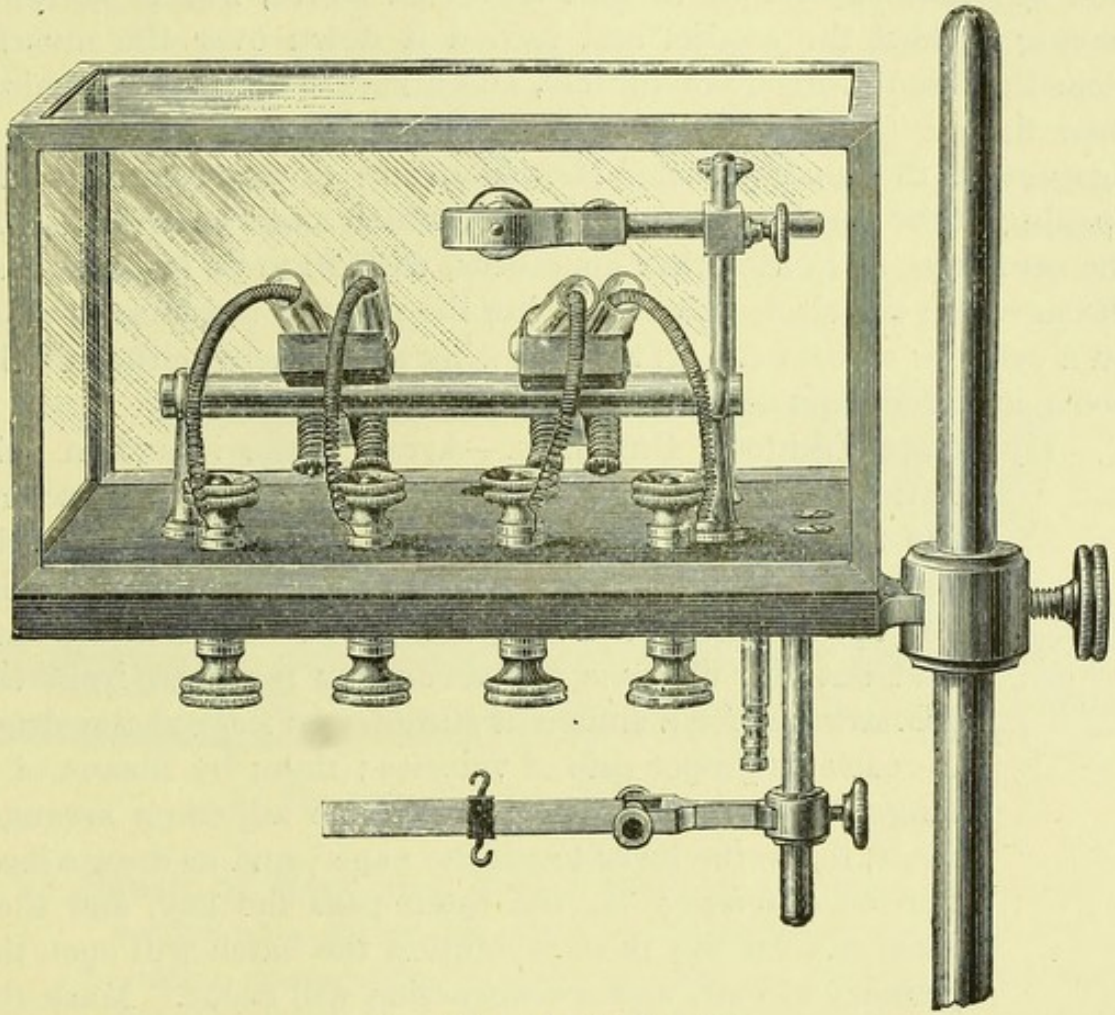

Fig. 108.—Moist Chamber.

Upon the same upright, slide (1) an electrode holder (not seen in Fig. 108), made very simply by fixing two copper wires in a small block of wood with the centre hollowed out, the wires being stretched across the hollow, and the ends fixed in the wood; they must be about $\frac{1}{3}$ of an inch apart, and are to be separated by a piece of cork. This wooden block is fixed upon a holder fitted with a collar and screw to move up and down the upright. (2) A brass rod, with the circular brass holder of a screw-clamp working easily in a collar at its end. The clamp holder can be fixed by a screw. A glass shade, large enough to cover the whole of the above, fits into a groove which runs round

the platform ¼ of an inch from the edge. When in use, pieces of blotting-paper, wetted with water or saline solution, are inserted beneath the shade, to keep the contained air moist; hence the apparatus is called a *moist chamber*.

Under the platform of the moist chamber is attached a metal screw collar apparatus, similarly capable of movement up and down the upright of the stand, to which is attached a fine metal spring and lever of light wood, capable of movement up and down about a fulcrum near the collar. Having prepared a nerve-muscle preparation in a manner similar to that described above, but in addition having divided the sciatic, and turned it down over the muscle, clear the femur entirely of muscle, and divide it at its lower third; then fix the femur in the clamp of the moist chamber, attach a ligature to the tendon (*tendo Achillis*), and carry it through the opening of the stage to the lever below, which may be weighted in the same way as in the other apparatus, with 10 to 20 grms.; place the nerve on the electrodes, and bring the point of the lever to write on a cylinder as before. In the following experiments either of the above arrangements may be used:

(1) **Single Induced Currents.**—Arrange the induction coil, battery and electrodes as described (p. 292), interposing the turn-over or kick-over key in the primary circuit. Cause the cylinder to revolve on the front axis, with the key open. Before allowing the lever to touch the drum, find out the distance the secondary must be from the primary coil for a sufficient stimulation; allow the drum to reach its proper rate of velocity; then, by means of a tangent screw, or some other delicate adjusting arrangement, make the lever touch the paper, and so draw a base line or *abscissa;* let the catch pass the key, and then close it. In the next revolution the catch will open the primary circuit, and a contraction will occur. Mark the exact period of excitation by allowing the cylinder to make nearly another revolution; close the key, and gradually advance the cylinder, until the catch touches it, then slightly raise the lever, and allow it to mark the paper. The interval between the entrance of the stimulus and the commencement of the contraction of the muscle is the **latent period.**

(2) **Faradization.**—Arrange the key in the secondary circuit, and arrange the apparatus for a tetanizing current (p. 294), using the magnetic interrupter. When the hammer is properly working, first of all draw an abscissa line, and then

allow the cylinder to revolve rapidly, and open the key. A curve of tetanus will be recorded.

(3) Records of **tetanus** curves with the spring (Fig. 104), and also with metronome, and with vibrating reed or vibrating tuning-fork (Fig. 109), may be taken in a manner similar to the above; the medium speed axis should be used. It is possible, by varying the length of the vibrating spring, to show the way in which a tetanus curve is built up. In all cases an abscissa line must first of all be drawn.

(4) **Tuning-Fork for making Rapid Electrical Contact.**—This consists of a heavy cast-iron stand with a slot, into which is fixed a large tuning-fork. A platinum wire projects vertically downwards from the lower prong of the fork, and at each vibration dips into a cup containing mercury and completes an electrical circuit. The current thus formed is taken to a small electro-magnet placed between the prongs of the fork.

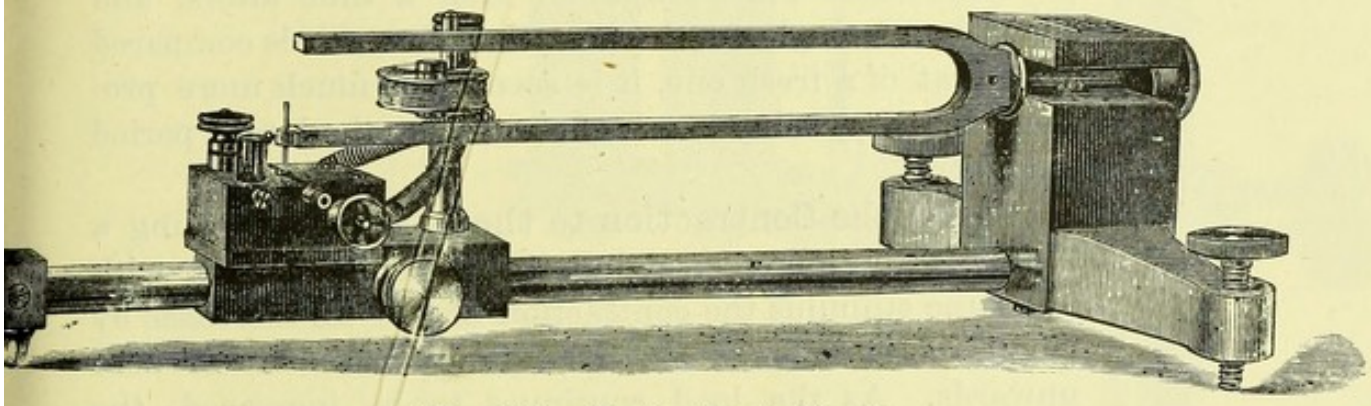

Fig. 109.—Tuning-Fork Stand.

(5) **Effects of Heat and Cold.**—Arrange the cylinder on the second axis, allow the lever in connection with a nerve-muscle preparation to mark on the smoked paper, and set the clockwork in motion; an abscissa line will be drawn. Interpose the kick-over key in the primary circuit, and by allowing the catch to open the key, record upon this line a single muscular contraction, and mark the latent period. Now cool down the muscle by filling a test-tube full of small pieces of ice, and bring it for ten minutes into close proximity to the muscle. Then set the cylinder in motion; and when everything is in readiness, remove the test-tube and close the key. On its next revolution the cylinder will open the key, and a muscular contraction will occur at the same point as before; the curve will be found to

be less sudden and more prolonged; the latent period must be marked, it is of longer duration. By filling the test-tube with water of gradually increasing temperature, a series of curves may be taken on the same line, which will show that up to a certain point the curve will be more sudden and of shorter duration.

(6) **Effects of Poisons.**—Inject to $\frac{1}{20}$ mgrm. of veratria into the posterior lymph-sac of a frog, and record the contraction of the gastrocnemius at various intervals after injection. It will be found that the full effect of this drug is to enormously lengthen the curve. The medium speed axis should be employed. The writing lever may not return to the abscissa line for several revolutious of the drum. Other poisons may be tried in a similar manner.

(7) **Effect of Fatigue.**—After using a muscle to demonstrate a single twitch, tetanus, and for other experiments, it will be found that the contraction after a time alters, and that when a single curve of a fatigued muscle is compared with that of a fresh one, it is seen to be much more prolonged, and possibly less high, and that the latent period is longer.

(8) **Relation of the Contraction to the Load.**—By loading a muscle with different weights, it will be found that with the same stimulus the contraction first of all increases by small increments as the load is increased from zero upwards. As the load continues to be increased, the increment diminishes, and finally gives place to a decrease. The initial increase of contraction is most prominent when its stimulus lies within a certain range of intensity.

(9) **Time Measurement.**—This is done by the vibrating tuning fork, or by a reed made to vibrate a definite number of times (25 to 200) in a second. The tuning fork of large size has on one prong a small style attached; and after the prong has been smartly tapped by means of a mallet or similar instrument covered with felt, the style, if applied to the revolving cylinder, will mark the number of times the fork vibrates in a second.

Another way is to place the tuning fork, or vibrating reed or spring, in a battery circuit, using one or two Bunsen's cells, and to allow its vibrations to be communicated to a small chronograph, which writes on the record-

ing surface. This instrument consists of a small electro-magnet. Each time the iron is magnetized it draws down a piece of metal, arranged on a frame in such a way that it can move to or from the magnet; at the other end of the frame is a small pedunculated hook, to which is fastened an elastic counterpoise. To the frame is fixed a style, capable of writing on a drum. Each time the rod vibrates, the current is made, the magnet draws down the piece of metal, and so the style makes a stroke on the smoked paper. At the break the elastic raises the style, and so on.

Another form of chronograph is seen in Fig. 110. The student having taken tracings of muscle contractions

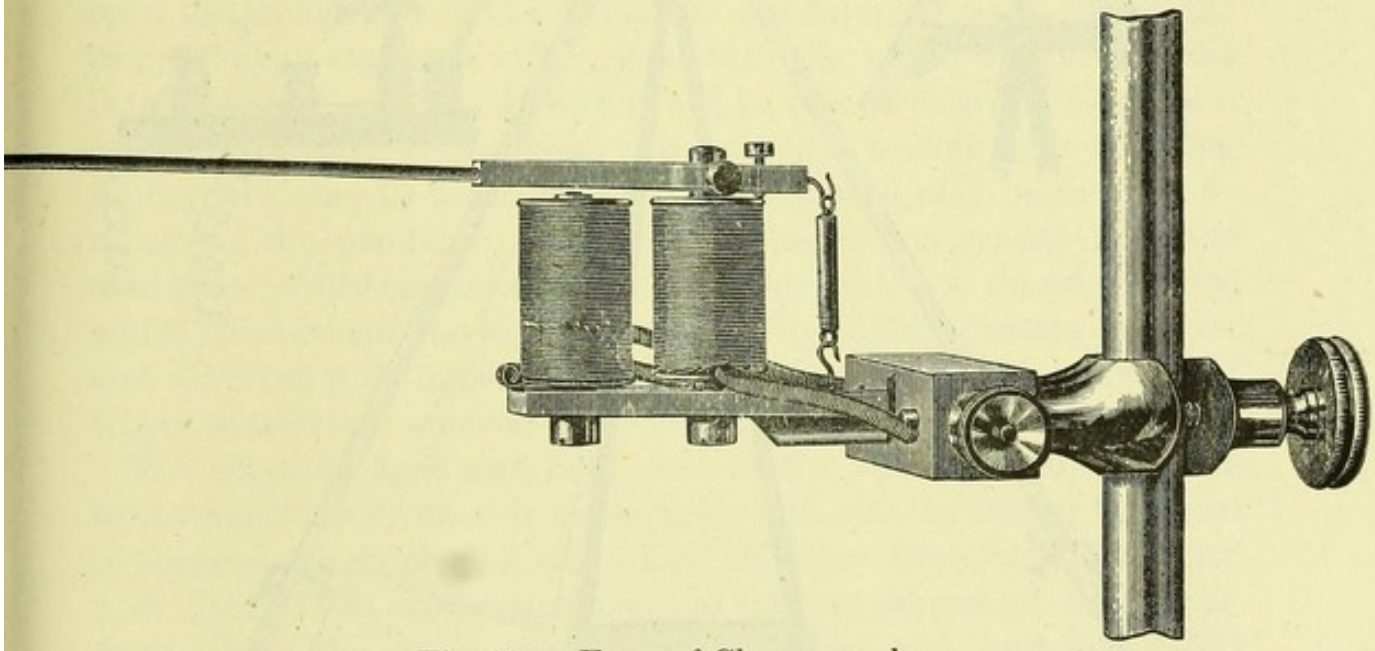

Fig. 110.—Form of Chronograph.

under various conditions, should employ one of the methods of time-marking, causing the writing-style of the time-marker to mark under the abscissa line. After the tracings have been varnished (with white hard varnish 1 part, and methylated spirit 2 parts) and dried, he should draw lines through the four points of the tracing, viz., (*a*) entrance of stimulus; (*b*) commencement of the curve; (*c*) highest point of curve; and (*d*) point where lever has reached the abscissa line again after its elevation; and also through the time-tracing below from the number of vibrations corresponding to *ab*, *bc*, *bcd*, and knowing the rate of vibrations, he can calculate the relative duration of (*a*) the latent period, (*b*) the period of contraction, and (*c*) the period of relaxation.

The Pendulum Myograph.—In this instrument (Fig. 111), the clockwork movement, which is frequently unreliable, is replaced by the force of gravity. The recording plate is attached to a pendulum. On this principle several instruments, differing somewhat in detail, have been constructed. The pendulum with the recording plate is fixed by a catch, which is capable of being moved certain distances along the arch through which the pendulum swings. When the arc is lengthened, the velocity is altered. In its swing the pen-

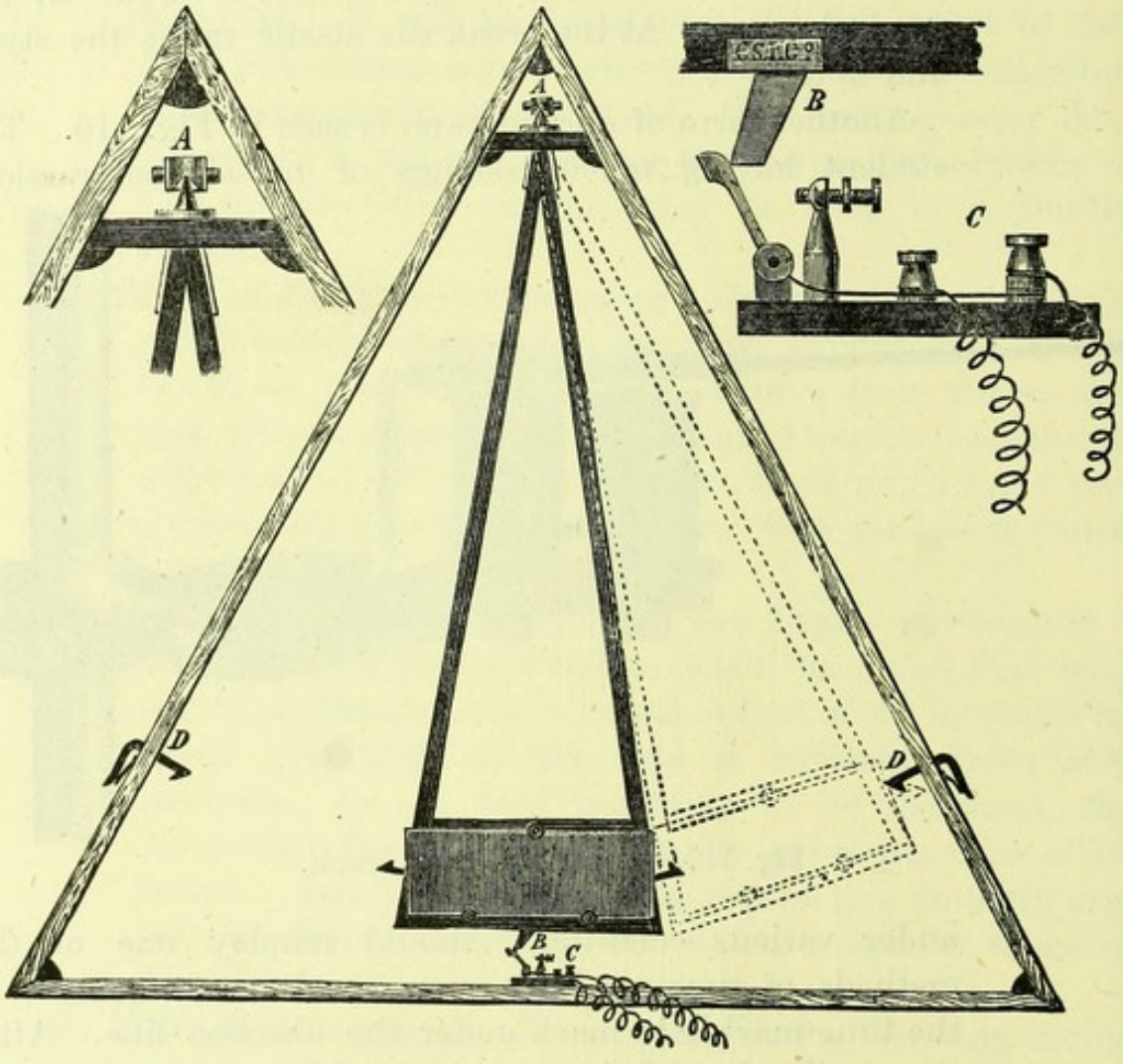

Fig. 111.—Pendulum Myograph.

dulum knocks over, with a catch (Fig. 111, *B*) attached to the middle of its lower edge, a trigger key (Fig. 111, *C*) placed in the primary circuit, and by this means the muscle of a muscle-nerve preparation gives a contraction. The preparation is arranged in a manner similar to that described above (p. 296) for the other kind of recording apparatus; but the frog apparatus is fixed in a collar to the upright of a firm stand, capable of being raised or lowered by means of a screw. The upright is fitted with a circular movement worked by a tangent screw at its base. The stand, with the

frog apparatus, is placed upon a firm table close to the myograph; this table can not only be raised and lowered, but is also capable of a circular horizontal movement.

Method.—Allow the pendulum to hang vertically. Arrange a nerve-muscle preparation on the table as above described. Cover the glass plate of the pendulum smoothly with glazed paper; having smoked it, replace the plate. Adjust the lever so that it barely touches the plate at its edge. Arrange a pair of electrodes under the nerve, interpose a key in the secondary current of an induction apparatus, and fix the trigger key (Fig. 111, *C*) at a convenient place in the battery circuit. Close the key in the secondary circuit (key 2), then raise the pendulum, and fix it in the catch to the right; see to the adjustment of the writing lever. Close the trigger key, then open the second key, and set free the pendulum. As it passes the trigger key, a stimulus will be sent into the nerve by opening the battery circuit, and a contraction will be recorded on the plate as it passes the lever. Underneath this tracing, a tracing of a vibrating tuning fork may be taken, and the latent period may be marked by returning the pendulum to its place, closing the second key, and then by carefully approaching the pendulum catch to the trigger key, which can be kept closed by the finger; by slightly raising the lever and allowing it to make a mark upon the paper, the exact point where the current entered the nerve is recorded.

The effects of **heat and cold**, and of the action of **poisons**, can be demonstrated with this myograph; and also, by taking two pairs of electrodes, and placing one a long distance from, and the other near the muscle, connecting them to the end screws of a reverser, from which the cross wires have been removed, and alternately throwing the current into one or the other pair of electrodes; the **effect** of having a **long or short piece of nerve stimulated** may be shown by the difference in the length of the curve. By having two trigger keys arranged at small distances, two coils, batteries, and electrodes, the **effect of two stimuli** acting one after the other may be shown.

The Spring Myograph.—Experiments should be done with the spring myograph (Fig. 112), in which the clockwork is replaced by the momentum imparted by a strong coiled spring. The recording surface is, as in the case of the pendulum myograph, a smoked glass plate; and in this instrument, as in the other, the glass plate in its course opens a trigger key, and a contraction similarly ensues, as the apparatus, induction coil, key and electrodes are arranged in an almost exactly similar way.

Note.—*Spring Myograph modified by Du Bois-Reymond.*—The most important features in this machine are seen in the engraving. To make the instrument ready for use the frame is moved to one side, and this movement compresses a short spring. When the catch holding it in this position is released by the trigger, the spring, which only acts for a short space, gives the frame and

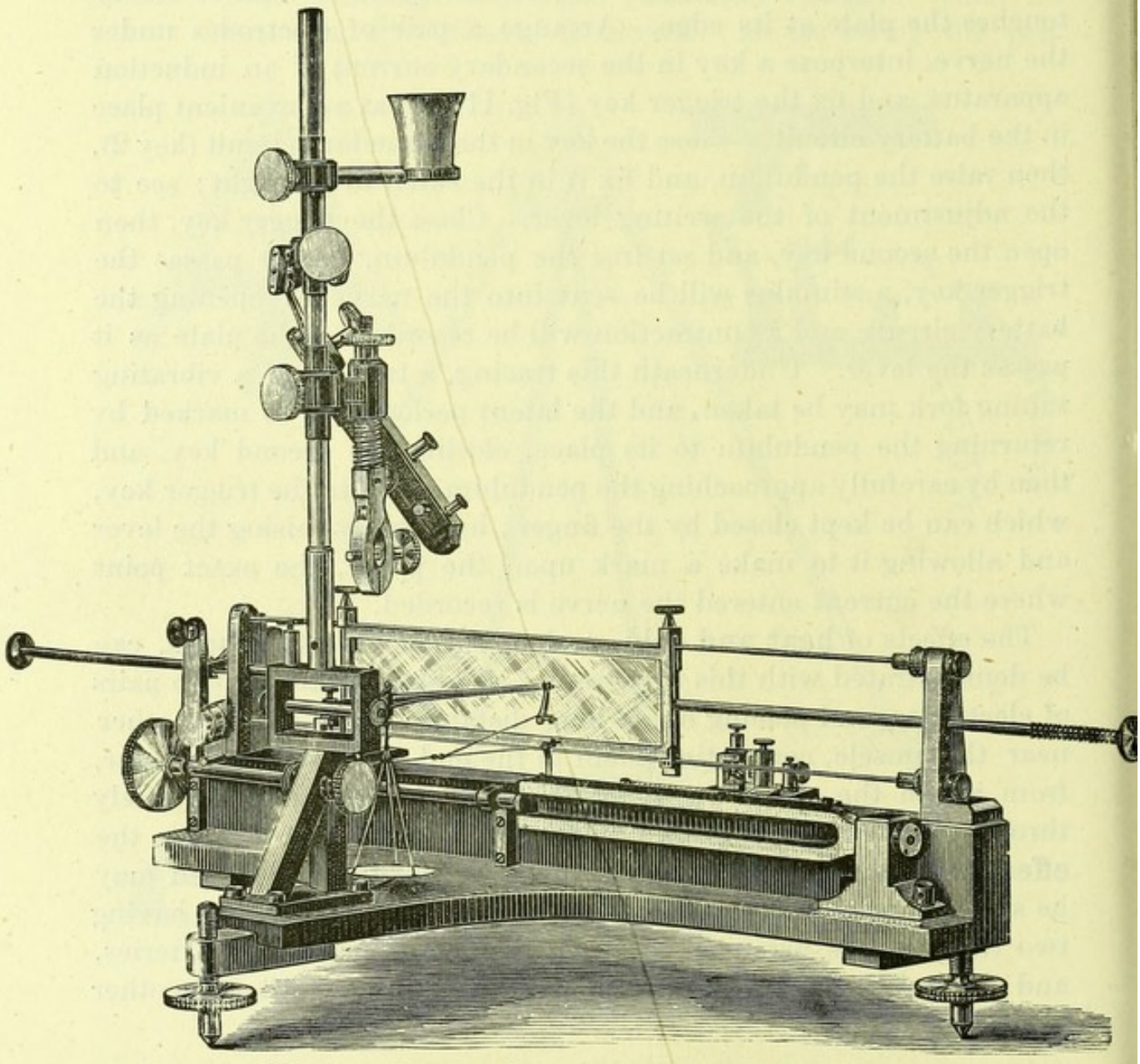

Fig. 112.—Du Bois-Reymond's Spring Myograph.

the glass plate a rapid horizontal motion, and the momentum carries the plate through the rest of the distance until it is stopped by the buffers. The friction of the guides (which are placed horizontally, and which consist of two steel wires) is very slight, and the velocity of the plate is, therefore, nearly constant. Two keys are knocked over by pins on the frame and break electric circuits; they are light, and do not offer any appreciable resistance to the

motion of the glass plate. The relative positions at which the circuits are broken can be altered by a convenient adjustment. A tuning-fork, vibrating about 100 per second, fixed to the base of the instrument, marks the time; its prongs are sprung apart by a block between their ends, and the same action which releases the glass plate also frees the fork by removing the block and allows it to vibrate; a writing-style then draws a sinuous line on the smoked surface of the moving glass plate. A muscle lever with a scale-pan attached also forms part of the instrument. There is a convenient arrangement for withdrawing the styles of both the lever and the tuning-fork from the surface of the glass plate. A vertical rod carries a clamp for holding the upper end of the muscle; an arrangement for warming the muscle is also carried by the rod.

MUSCLE CURRENTS.

Arrange a Thomson's galvanometer (Fig. 113) in a dark chamber, levelling by means of the screws *s*, and place the scale (Fig. 114) at about 3 feet *east and west* facing it. Light the paraffin lamp, remove the glass cover (Fig. 113, *l*), and, after having set the mirror free by raising the screw adjustment at the top of the instrument, replace it; adjust the lamp so that the light falls well on it. By means of the magnet (Fig. 113, *m*), adjust the mirror until it throws its light upon the zero of the scale. To the binding screws of the galvanometer attach the screws from the shunt (Fig. 115), and, for the sake of practice, allow a very weak current to pass from a Daniell's battery; by attaching wires from the battery to the shunt, with the plug in the hole marked $\frac{1}{999}$, the needle will be deflected, as indicated by the movement of the spot of light on the scale; the light will probably move in the same direction as the current. Prepare a pair of non-polarizable electrodes, and connect them with the shunt; the plugs being in the shunt, place the electrodes so that their plugs touch: on opening the shunt there will be no deflection.

(1) Now take a pithed frog, and having dissected out the sciatic nerve, fix the femur in a cork plate by means of a pin, and having attached a ligature to the tendo-Achillis, tie it firmly to a hook, and stretch it slightly; remove the remainder of the frog from the gastrocnemius thus prepared; place the electrodes, having inserted the plugs in the shunt, so that one touches the middle, and the other one end of the muscle; a current from the muscle from the equator to the pole can be demonstrated, called the **current of rest**, or **natural muscle current**; now open

the plug $\frac{1}{9}$, the light will quickly pass off the scale; reinsert the plug, and remove another, so as to allow less of the muscle current to pass through the galvanometer—say, $\frac{1}{1000}$; then note the amount and direction of the current, from the direction of the deflection and the degrees of the scale the light has passed over. Similarly the position of the electrodes may be altered, the variations in the currents noted, and the general laws demonstrated.

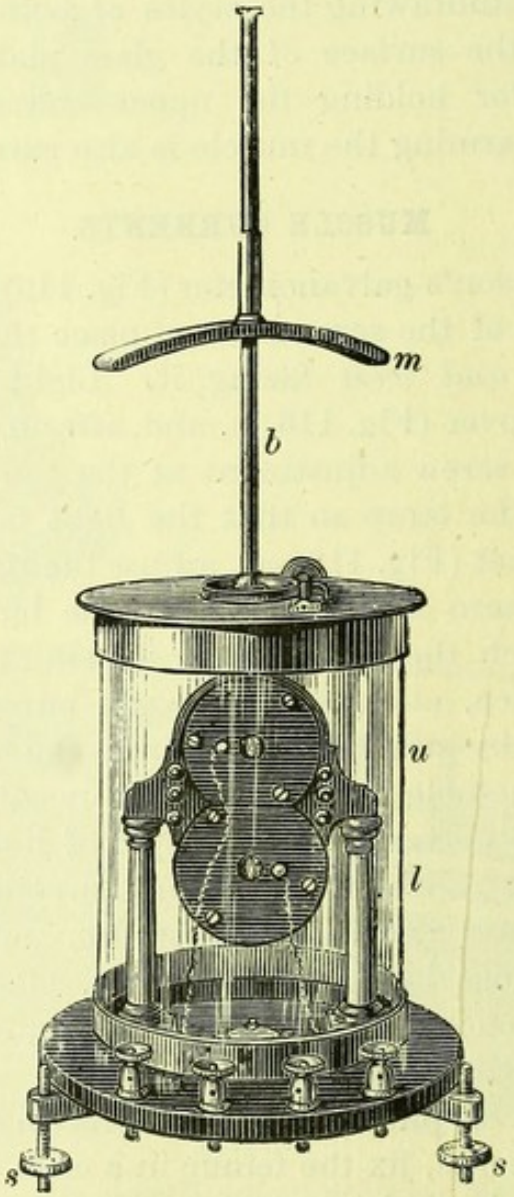

Fig. 113.—Thomson's Galvanometer.

(2) For the purpose of showing **the negative variation** of the muscle current when the muscle enters into contraction, arrange under the nerve a pair of electrodes connected with an induction coil (A, G, B, C); the rest of the apparatus being as before. But it is first of all necessary to neutralize the muscle current at the points on

the surface of the muscle where the electrodes are placed, by sending in the opposite direction a weak current from a Daniell's battery, as in the diagram (Fig. 116); the amount which it is necessary to use can be regulated by a rheochord or compensator L, and

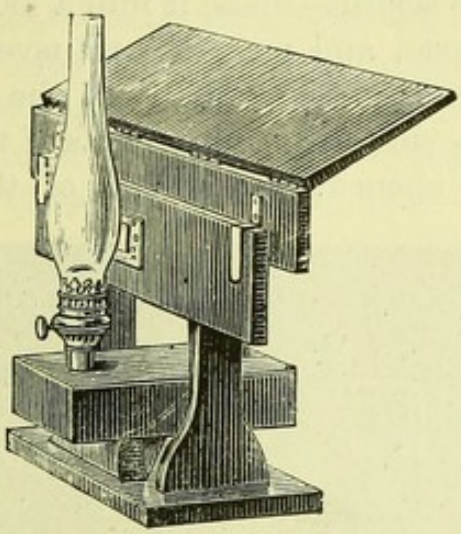

Fig. 114.—Lamp and Scale for Thomson's Galvanometer.

the direction of which may be regulated by reverse K. Having exactly neutralized the current, the key G is opened, and the muscle is stimulated; the galvanometer needle is then seen to move in the opposite direction to its former movement; this is the **negative variation** of the muscle current, or **current of action**.

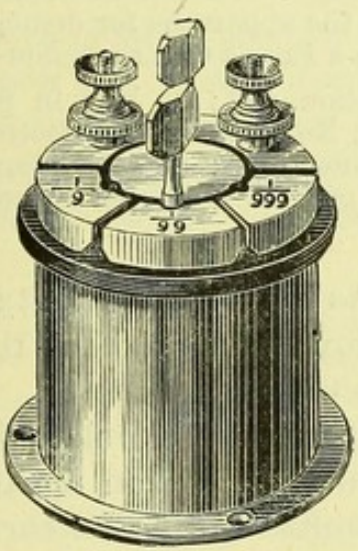

Fig. 115.—Shunt for Galvanometer.

(3) Arrange the apparatus in exactly the same way as in 1; but, instead of the gastrocnemius, use the excised heart of a frog. Ascertain the strength of the muscle currents present. In removing the heart, the excision is almost

certain to set up a current of rest. With the electrodes at the apex and base of the heart, when the heart contracts, notice the negative variation of the muscle current.

(4) Repeat the experiment, but use instead a thick nerve-trunk —*e.g.*, the sciatic—make it into a loop, place the loop on one electrode, and put both transverse sections on the other electrode. The shape of the clay plugs may be altered to suit the requirements of the case. When the key I is opened, the needle of the galvanometer is

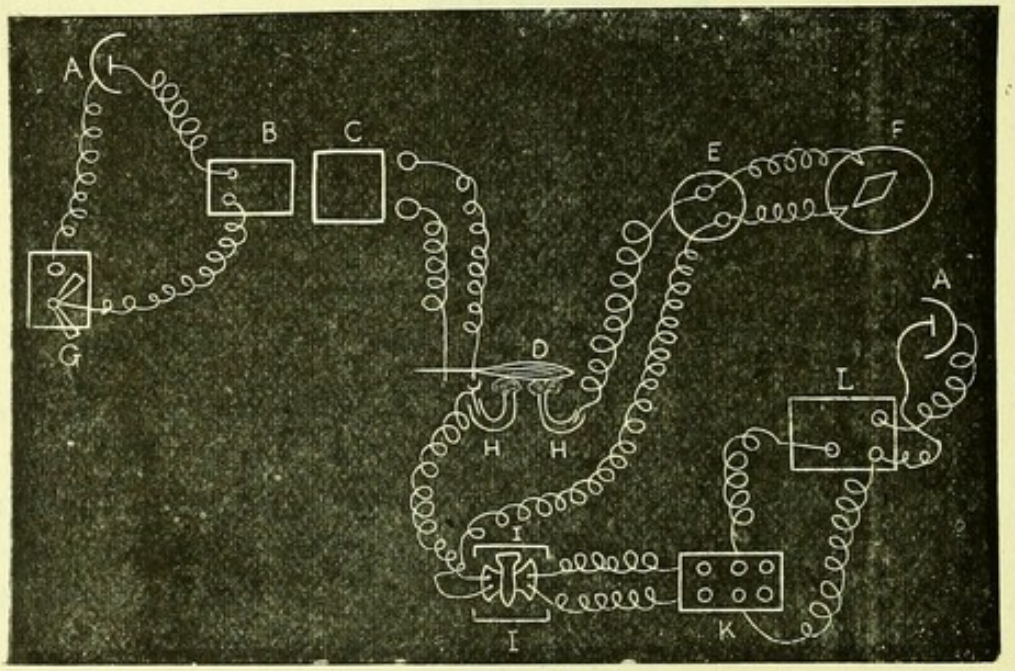

Fig. 116.—Arrangement of the apparatus for demonstrating Muscle Currents in a Frog's Gastrocnemius.

A, Battery; B, C, Induction coil; G, Key in primary circuit; D, Nerve-muscle preparation; H, H, Non-polarizable electrodes; I, Plug key; E, Galvanometer shunt; F, Galvanometer; A, L, K, Arrangement by means of which a current from a battery may be regulated by compensator L, to neutralize the muscle current.

deflected. As the nerve current is small it is not necessary to employ the shunt, but the whole of it may be sent through the galvanometer.

(5) Repeat 4, but arrange to stimulate the nerve with tetanizing currents from an induction apparatus; but, first of all, as in 2, neutralize the nerve current in the same way, then stimulate the nerve, and notice the deflections of the galvanometer-needle in the opposite direction to the original current. (N.B.—This negative variation may be so slight as not to deflect the galvanometer needle.)

(6) Repeat the experiment 1 to 5, but use in place of Thomson's galvanometer either an ordinary **electrometer**, or,

much better, a **capillary** or **mercury electrometer.** In the latter case the variations of the thread of mercury are observed with a microscope.

(7) The **Rheoscopic Frog.**—Prepare two nerve-muscle preparations, in the one case exposing the sciatic nerve throughout its length, and removing the skin from over the gastrocnemius only, but in the other case removing the leg with a long length of nerve. Insert the electrodes beneath the sciatic nerve of the first preparation; place this on a glass plate, in order that it may be insulated. Place the sciatic nerve of the second preparation over the thigh muscles of the first, and excite the muscles of the first with a single induced current; the muscles of the second will contract. Repeat with a series of shocks; the second preparation will be thrown into tetanus, as well as the first. Show that this is not due to escape of the current by ligaturing the nerve of the second preparation, when the contraction no longer takes place. It is caused by the contraction of the muscles of the first, producing a negative variation of their current (p. 307). This acts as a single stimulus to the nerve of the second, and so causes a contraction.

(8) Instead of passing a current through the nerve-muscle preparation, the nerve of the second may be dropped upon the muscle of the first preparation in such a way that one part of it falls upon the equator, whilst another part falls upon the muscle near its insertion into the tendon, or upon a transverse section of the muscle. The instant that the nerve falls upon these two points, the muscle of the second preparation will give a single contraction.

(9) The experiment may also be varied by allowing the nerve of the limb to rest in a curve upon the exposed heart of the frog; at each systole of the heart the muscles of the limb will contract.

ELECTROTONUS.

1. Prepare a nerve-muscle preparation, and remove it from the body, taking care that the sciatic nerve is uninjured, and very long. Place the preparation in a moist chamber, and arrange the nerve over two pairs of non-polarizable electrodes; connect one pair of electrodes with an induction coil, and arrange for single shocks; connect the other pair with a rheochord, the binding screws of which

are also joined to the end screws of a reverser, to the middle screws of which the wires of one or more Daniell's elements are connected. Find out the exact minimal current which will cause a contraction, and then move the secondary coil a little farther away from the primary. On making or on breaking the primary current there will be no contraction; but if now a descending galvanic current, which is called the **polarizing** current, be sent into the nerve, an induction current weaker than the normal minimal will cause a contraction. This will also occur for some time after the galvanic current has been shut off. Similarly, it may be shown that if the current be ascending, an induced current stronger than the minimal will be required to produce a contraction; in like manner, the effects of a series of shocks may be shown, plus an ascending or descending continuous current. So we see that the irritability of the nerve is increased during the passage of a constant current in a descending direction, and diminished if it be in a contrary direction. If the relative position of the electrodes of the induction and polarizing current be reversed, an exactly opposite effect occurs in all particulars.

But the irritability is always increased in the neighbourhood of the kathode, constituting the condition known as **kath-electrotonus**, and diminished in the neighbourhood of the anode, constituting the condition known as **anelectrotonus**. This alteration in the irritability is the effect of the passage of the constant current, which is **non-apparent**, and must be remembered in connection with the contraction at the make or at the break of the current, or at both, which takes place, and which is sufficiently evident.

2. **Paradoxical Contraction.**—Make a nerve-muscle preparation, and follow the two divisions of the sciatic nerve to the two heads of the gastrocnemius, divide the nerve above the junction of its branches, also divide one of the branches at the entrance into the muscle. Place the preparation upon a glass plate, and stimulate the central end of the divided nerve with a galvanic current. The gastrocnemius will contract. This contraction is due to the alteration of the irritability of the cut branch, produced by the passage of the galvanic current acting as a stimulus to the part of the nerve in the same sheath with it.

OTHER PROPERTIES OF MUSCLE.

1. **Elasticity.**—Prepare the gastrocnemius attached to the femur, clamp the femur, attach the gastrocnemius tendon to the lever of the moist chamber, and load the lever with a 10-grm. weight. Allow the lever to mark on a recording cylinder, then

load with 20 to 100 grms. It will be found that the extensibility—relatively large at first—gradually diminishes for equal increments of weight. On removing the weights, the lever will return to the same point on the paper from which it started, indicating the **perfect though slight elasticity.**

2. Repeat the experiment with a tetanized muscle, and compare the results. It will be seen that the **extensibility is greater in the contracted muscle, but that the elasticity is not so perfect.**

3. **Reaction.**—Remove from a pithed frog a gastrocnemius which has been perfectly freed from blood, cut it across with a sharp knife, and apply blue and red litmus paper to the ends. It will be found that there will be a bluish mark on the red litmus.

4. **Transparency.**—Take a flat muscle from a pithed frog—*e.g.*, the *mylohyoid* or *sartorius*—and as soon as possible place it on a slide in saline solution. Examine with a $\frac{1}{4}$ inch, and focus through the muscle some vessel underneath the fibres. It will be found quite clear, and so prove the transparency of living muscle. On entering into *rigor mortis* this property disappears.

CHAPTER III.

THE FROG'S HEART.

ANATOMICAL CONSIDERATIONS.

1. Open the abdominal cavity of a pithed frog by a longitudinal incision, avoiding the large veins in the middle line; cut through the middle of the sternum and pin out the arms upon a **frog-board**, which is a piece of thin wood, about 6 × 4 inches, covered with sheet-cork on either side; pass a thick glass rod down the œsophagus, open the pericardium, and note the following points:

The two auricles, one on either side; and
The conus arteriosus on the right side, dividing into the two aortæ.

2. Lift up the apex carefully with a pair of fine, blunt-pointed forceps, and the fine ligament or frænum, connecting the dorsal aspect of the ventricle with the pericardium, comes into view. Tie a fine ligature to the frænum (**frænum ligature**), and then sever it from the pericardium. By gently pulling on the frænum ligature the heart is raised and turned backwards, exposing its posterior surface, the apex of which pointing upwards is composed of the ventricle, the auricles being below it; below the auricles (in this position of the heart) and behind is the sinus venosus; at the junction of the sinus with the right auricle, just above the auricular ventricular groove, is seen a curved white line. The sinus venosus is seen to be formed by the confluence of the inferior vena cava, the two hepatic veins, and the two superior venæ cavæ.

3. Note the sequence of the rhythmic contractions, of the sinus venosus, auricle, and ventricle.

4. **The Ganglionic Centres.**—The vagi run in company with the superior venæ cavæ, and on reaching the sinus venosus split into a plexus, which is beset with ganglion cells (**Remak's ganglia**). Some of the fibres are gathered together and pass on

into the septum auricularum, where there are again many ganglion cells (**Bidder's ganglia**). These fibres pass down in the septum to the upper part of the ventricle, where there are secondary ganglionic masses, particularly on the dorsal aspect.

5. **The Inhibitory Centre.**—Turn up the ventricle and apply electrodes to the white line above mentioned, stimulate with the interrupted current for a second or two, and notice that the heart stops in diastole, but soon goes on beating again.

6. **Dissection to expose the Vagus.**—The easiest way to do this is to dissect away the integument over the scapula, cut through all the muscles attached to it, keeping close to the bone

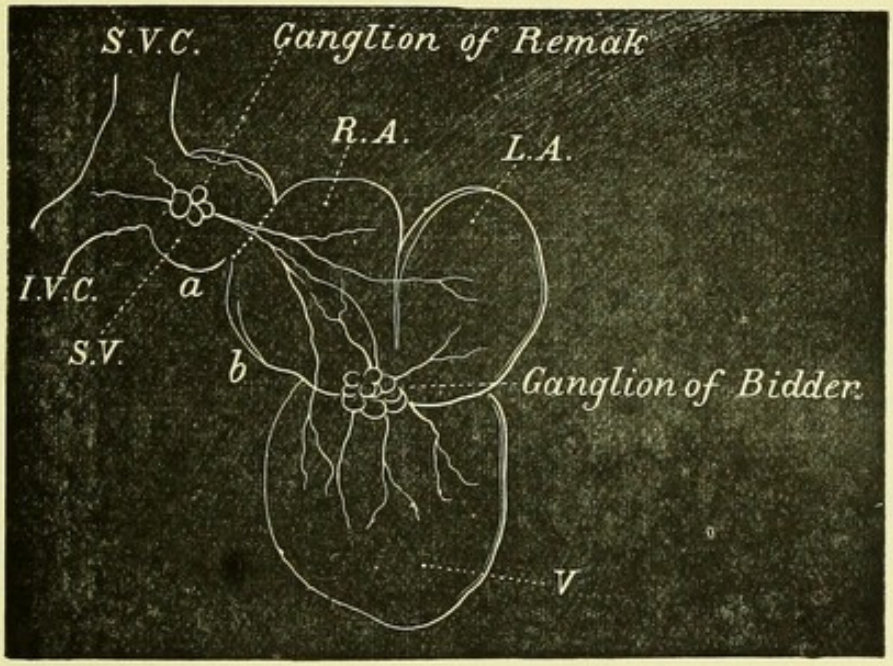

Fig. 117.—Ganglia of Frog's Heart.

to divide the brachial nerves and vessels and so to remove the scapula with the fore-limb attached. Close under the spot where the aorta of that side gives off the carotid and axillary vessels lies the cornu of the hyoid bone; to this is inserted a thin strip of muscle which can be traced upwards and backwards to its origin on the petrous bone: this is the inferior petrohyoid. On this muscle lie **four nerves**; the most anterior passes down for a short distance, and turns sharply forwards and upwards; towards the tongue is the **glosso-pharyngeal**; the next, a slender nerve, passing down the middle of the muscle to be distributed to the larynx is the **laryngeal branch** of vagus; on the posterior border of the muscle lies the **vagus,** which passes down over the root of the lung to the sinus venosus. The nerve generally lies under some pigmented veins and

fascia. The **hypo-glossal** nerve crosses the vagus and proceeds, internally, below the glosso-pharyngeal to end in the muscle of the tongue near the middle line.

7. **Stimulate the Vagus.**—(*a*) With a very weak interrupted current, and with the arrangement known as Helmholtz modification. Notice that the beat is slow by lengthening the diastole. (*b*) With a strong current the heart stops, after one beat, in diastole.

8. **Intrinsic Nervous Mechanism.**—Excise the heart with a pair of sharp scissors, leaving behind the sinus venosus, place the excised auricles and ventricle into ½ per cent. salt solution.

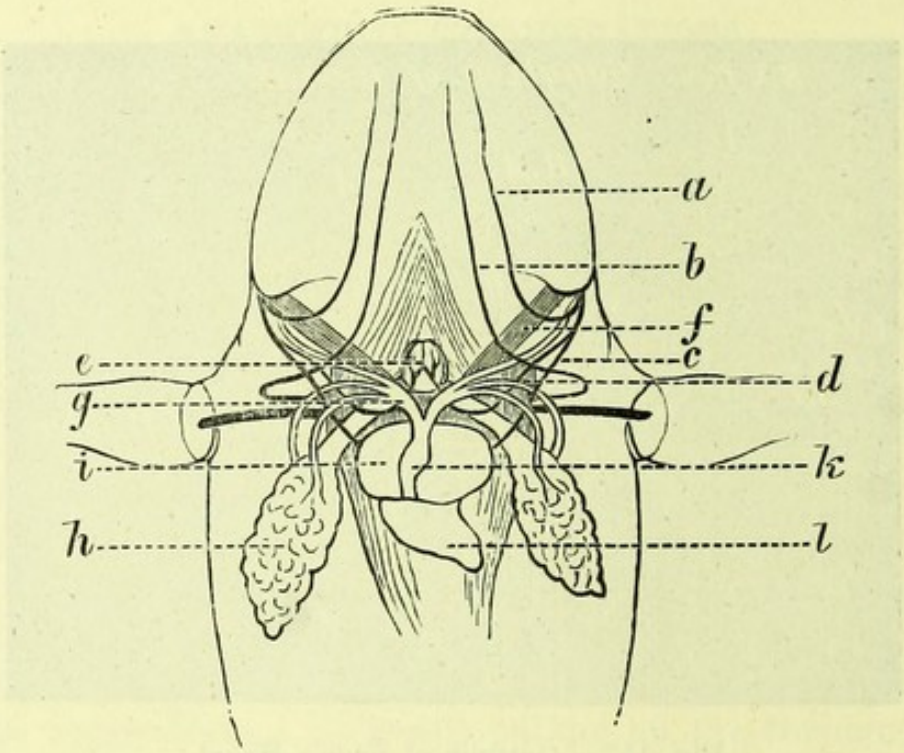

Fig. 118.—Dissection showing position of Vagus in the Frog.

a, Glosso-pharyngeal nerve; *b*, Hypo-glossal nerve; *c*, Vagus; *d*, Laryngeal nerve; *e*, Larynx; *f*, Petrohyoid muscle; *g*, Aorta; *h*, Lung; *i*, Auricle; *k*, Bulbus arteriosus; *l*, Ventricle.

Notice that the sinus goes on beating as before, but that the excised heart stops for a time and then goes on again, especially if stimulated; the rhythm, however, is different from that of the sinus.

9. Separate the auricles from the ventricle; both go on beating, but with independent rhythm.

10. Separate the upper third of the ventricle from the lower two-thirds. The latter will no longer contract rhythmically, but will respond to a single stimulus with a single beat, the latent period being very long.

11. **Stannius' Experiment.**—Pass a ligature between the auricle and sinus venosus below, and between the aortæ and venæ cavæ superiores above. Tie it tightly, so that the sinus is separated by the ligature from the rest of the heart. The heart stops in diastole, but the sinus beats naturally. On stimulating the flaccid heart single, and sometimes a succession of, beats may be obtained. If now the heart be separated by a clean incision along the line of the ligature, it will resume an independent rhythm to that of the sinus.

12. **Action of Poisons.—Muscarin or Pilocarpine.**—Place the excised heart in neutral saline solution, with a trace of muscarin or pilocarpine solution. It will become quite motionless in diastole. **Atropin.**—Transfer the motionless heart to a ·2 per cent. solution of atropin in neutral saline solution. Notice the gradual return of the beat. Atropin is the antagonist of muscarin and pilocarpine.

13. **Further action of Atropin.**—Inject under the skin of the back a few drops of atropin solution, 1 per cent. After about ten minutes pith the frog and make a vagus preparation. Stimulate the vagus; no inhibition follows, even with the strongest currents. Stimulate the inhibitory centre, and again no stopping of the beat results. The vagus nerve-endings are paralyzed. Stannius' experiment, however, succeeds as with the normal heart.

14. **Frog-Heart and Rheoscopic Limb.**—Prepare the hind-leg of a vigorous frog, together with a long length of uninjured sciatic nerve, and in the same pithed frog expose the heart and open the pericardium. Arrange the frog on a glass plate, and also the limb on another, then allow the nerve to fall upon the ventricle. Each time the ventricle contracts, a contraction occurs in the limb.

GRAPHIC METHODS.

For this purpose a hollow cylindrical box, about 3 inches long and 1 inch in diameter, is fixed upon a metal support. The box is provided with two metal tubes, by means of which water at various temperatures may be passed through it by attaching to the metal tubes gutta-percha tubes, the one passing from a vessel fixed on a stand at some distance above the frog-box, and the other similarly fixed somewhat below. The lever is thus made: a glass rod, of the thickness usually employed as a stirrer, is taken, and with a blow-pipe flame it is softened sufficiently to allow of its being drawn out at the softened part to great fineness: the fine part is then broken

at a point about 5 inches from the unaltered glass tube, which is now similarly drawn out on the other side, leaving a knob of glass between the two thinner parts. On this side all the thinned part is removed, and the glass now remains with a thin arm about 5 inches long. A square piece of cork is now passed along the thin glass to the knob, and through this a fine needle is passed. The needle can be adjusted in bearings which are fixed to the edge of the box. A second piece of cork is passed along the lever arm, and is adjusted and cut so that its point, directed downwards, can rest upon the ventricle of the heart. After these corks have been put in place, the writing end of the lever may be made by allowing the extremity to be softened for a few seconds in the flame of a spirit-lamp. The frog heart-box can be adjusted to the recording cylinder, and for the purpose of recording the contraction the cylinder should revolve slowly.

(1) Having exposed the heart of a pithed frog, tie a ligature to its frænum, and, cutting through the vessels, lift it by the ligature to the heart-box; having first allowed water at about 10° C. to pass through it, moisten the heart by placing a little serum on the top of the box by means of a capillary pipette. Adjust the lever so that the cork rests well upon the ventricle, and the writing lever marks on the recording drum record-tracings of the contractions at various temperatures, cooling the water down by means of ice to 1° C., and then raising it by increments to 20° C., and compare the tracings.

(2) If, instead of a heart beating in the ordinary way, a *Stannius' preparation* (p. 321) be substituted, the heart may be stimulated by weak induction shocks, the apparatus being arranged so that the electrodes just touch the ventricle; and with a key in the secondary circuit, and the kick-over key in the primary circuit, definite regular contractions may be compared, and the effects of temperature in altering the rapidity and the strength of the contraction seen, as well as the latent period recorded.

(3) **Endocardial Pressure.**—A large frog (*Rana esculenta*) is taken, and the heart is exposed in the usual manner, the pericardium opened, the frænum ligatured, and the heart turned over by the ligature. A cut is made into the bulb, and by this means a double or perfusion cannula is passed into the ventricle, a ligature is passed round the heart, and the cannula is tied in tightly. The vessels are then divided beyond the ligature, and the cannula, with the

heart attached, is removed. To one stem of the cannula a tube is attached, communicating with a reservoir of a solution of dried blood in ·75 saline solution, and filtered, which is capable of being raised or lowered in temperature by being surrounded by a metal box which contains hot, cold, or iced water. Attached to the other end is a similar tube, which communicates by a T piece with a small mercurial manometer, provided with a writing style, and also with a vessel into which the serum is received. The apparatus being arranged so that the movements of the mercury can be recorded by the float and the writing style on the slowly revolving drum, and

Fig. 119.—Kronecker's Perfusion Cannula, for supplying Fluids to the interior of the Frog's Heart.

It consists of a double tube, one outside the other; the end view is shown in the engraving. The inner tube branches out to the left; thus, when the ventricle is tied to the outer tube of the cannula, a current of liquid can be made to pass into the heart by one tube and out through the other.

after some serum has been allowed to pass freely through the ventricle, both tubes are clipped, the second one beyond the T piece, and the alterations in the pressure are recorded.

(4) The effects of **fluids at various temperatures** and

(5) Of **poisons** should similarly be recorded in the manner indicated above.

(6) **Roy's Tonometer.**—By this apparatus the alterations in volume which a frog's heart undergoes during contraction are recorded by the following means: A small bell-jar, open above, but provided with a firmly fitting cork, in which is fixed a double cannula, is adjustable by a smoothly ground base upon a circular brass plate, about

2 to 3 inches in diameter. The junction is made complete by greasing the base with lard. In the plate, which is fixed to a stand adjustable on an upright, are two holes, one in the centre, a large one about one-third of an inch in diameter, to which is fixed below a brass grooved collar, about half an inch deep; the other hole is the opening into a pipe provided with a tap (stopcock). The opening provided with the collar is closed at the lower part with a membrane of animal tissue, which is loosely tied by means of a ligature around the groove at the lower

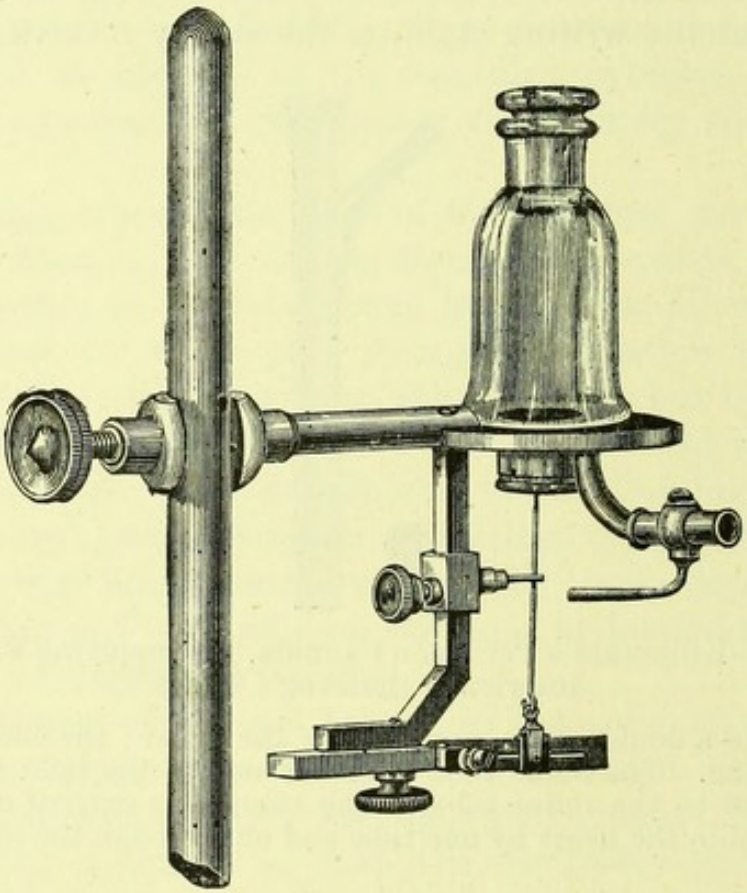

Fig. 120.—Roy's Tonometer.

edge of the collar. To this membrane a piece of cork is fastened by sealing-wax, from which passes a wire, which can be attached to a lever, fixed on a stage below the apparatus.

When using the apparatus, fix the bell-jar by means of lard, drop a little glycerine into the collar closed by membrane, and fill the jar with olive oil. Now prepare, in the way above described, the heart of a large frog, tie in the cannula, which is, as before mentioned, fixed in the cork; the tubes of the cannula communicating with the reservoir of serum on the one hand, and with a vessel to

contain the serum after it has run through on the other. Pass the cannula with heart attached into the oil, and firmly secure the cork. Now open the tap, raise the membrane a little, and allow a few drops of the oil to pass out; shut the tap, and let go the membrane. By these means the lever will be found to be adjusted to a convenient elevation. Allow the lever to write on a moving drum, pass serum through at various temperatures, and compare the tracings. After a short time the heart will stop beating; but two wires are arranged, the one in the cannula, the other projecting from the plate in

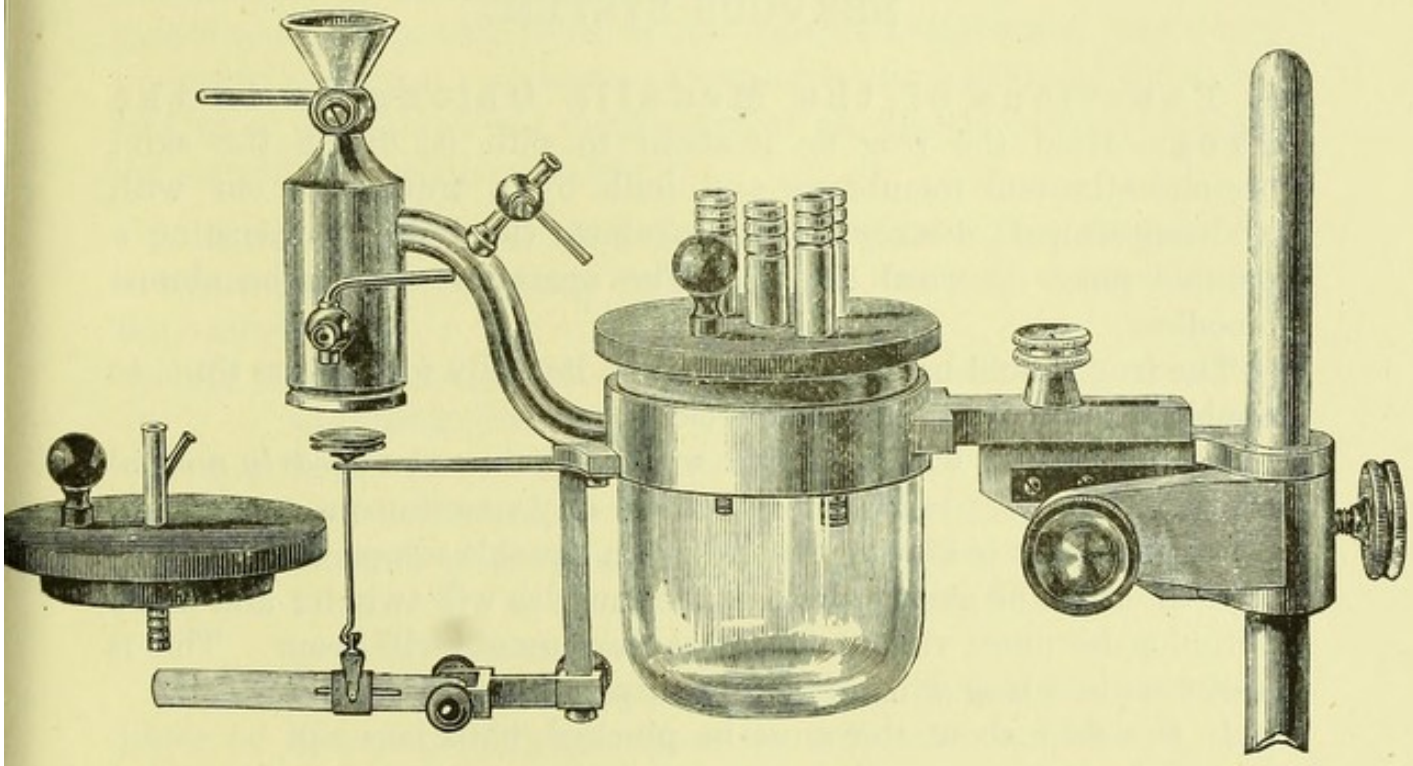

Fig. 121.—Gaskell's Tonometer.

such a way that the heart can be moved against them by shifting the position of the bell-jar a little. The wires act as electrodes, and can be made to communicate with an induction apparatus, so that single induction shocks can be sent into the heart to produce contractions, and if need be, by means of the trigger key, at one definite point in the revolution of the recording cylinder.

(7) **Gaskell's Tonometer.**—Repeat the experiment, but use instead of Roy's, Gaskell's tonometer (Fig. 121), which is on much the same principle. It has, however, the position of the glass containing the heart reversed.

CHAPTER IV.

NERVOUS SYSTEM.

1. **Functions of the Medulla Oblongata in the Frog.**—Hold the frog as if about to pith it, divide the skin, occipito-atlantoid membrane and bulb by a transverse cut with a sharp scalpel; destroy the brain above the bulb by thrusting a pointed piece of wood into it. The operation should be almost bloodless.

The frog should be allowed to rest on its belly for a short time, to enable it to recover from the shock.

Before long it will be found to have assumed a *nearly* normal attitude. It does not, however, make any spontaneous movement, provided that it is kept moist and at an equable temperature.

If the flank be gently stroked, the muscles will twitch; and if the stimulus be more violent, bilateral movements will occur. This is best seen in a frog which is freely suspended.

If the skin about the anus be pinched, both legs will be simultaneously drawn up.

2. **Functions of the Roots of the Spinal Nerves.**—Divide the skin along the back of a frog, the brain of which alone has been destroyed, as in the previous experiment. Separate the muscles of the back, so as to expose the arches of the vertebræ, and cut them away carefully with a pair of blunt-pointed scissors. The roots of the nerves will then be seen within the spinal canal. Expose the roots of the eighth, ninth, and tenth nerves, taking the greatest care not to touch them, by completely removing the surrounding structures. The posterior roots will then be seen to be the larger and the more superficial; they conceal the anterior roots. Select the largest of the roots now visible—it is that of the ninth nerve—and pass a fine silk ligature round it without touching it more than is necessary. Tighten the ligature. At the same

instant movements will be noticed in some part of the body of the frog.

3. Cut the nerve between the ligature and the cord; movement will again take place.

4. Place the proximal portion of the divided nerve upon a pair of electrodes in connection with a Du Bois-Reymond's induction apparatus; decided movements will occur on the passage of a current;

5. Whilst no such movements are seen when the distal extremity of the nerve is stimulated in the same manner.

6. Cut away the posterior root, and repeat the preceding experiments with the anterior root; movements will occur as soon as the root is touched, as well as when the ligature is tightened, and when the nerve is divided. Tetanus ensues upon stimulation of the distal portion of the nerve; but the passage of an electric current through the central end produces no result.

7. **Functions of the Spinal Cord.**—In a preparation in which the brain has been destroyed, and the cord divided below the medulla:

(*a*) The reflex function of the cord may be shown by irritating the surface of the skin by means of small pieces of filter paper dipped in acid, and placed in various situations; contraction of certain muscles usually follows for the purpose of getting rid of the irritation. The groups of muscles are as a rule regularly brought into action when particular parts are irritated.

(*b*) If the irritation occur in one leg, and that leg be prevented from moving, the other leg will act; but after a time general contraction may occur.

(*c*) If the irritation be very great, or if the frog be under the influence of strychnia, general convulsions may occur.

APPENDIX.

ON THE PRESERVATION OF NORMAL AND PATHOLOGICAL MUSEUM PREPARATIONS.

THE only satisfactory method of making permanent preparations of entire organs or tissues is to obtain the specimen whilst it is quite fresh. When this can be done it is placed in a zinc or earthenware basin, and a small stream of fresh water is allowed to flow upon it for twenty-four hours. At the end of this time it is deprived of the blood which it contains by gently squeezing along the course of the main vessels. It is then dissected in such a manner as to show the especial features for which it is preserved. It will be found that the dissection is more readily and cleanly performed at this stage than after the preparation has been placed in spirit. It is then put back into the basin, and the water is allowed to flow over it until the greater part of its colouring matter has been washed away. In cold weather this is readily effected, but in summer it must be carefully watched to prevent decomposition setting in. The amount of water which flows over the specimen is of less importance than its constant renewal.

As soon as the washing is complete, which will usually be in forty-eight to seventy-two hours after its commencement, the preparation should be suspended by means of silken threads in the glass jar in which it will eventually be preserved. A mixture consisting of half methylated spirit and half water is then poured over it. The bottle should afterwards be conspicuously labelled and set aside, the spirit being changed as often as it becomes discoloured. In dealing with large numbers of specimens it will be found convenient to place in each jar an unground microscopic slide or other slip of glass, upon which a reference to the specimen has been made by means of a writing diamond, details being at the same time entered in a book kept for the purpose. As soon as the spirit in which the specimen is immersed remains clear, the final dissection should be made under weak spirit, all loose pieces of connective tissue being removed with a pair of curved scissors, the muscles being carefully cleaned, and the various points of interest being

demonstrated to their greatest advantage. The preparation is then suspended in a mixture of proof spirit and water in the proportion of one part of spirit to two of water. Proof spirit is employed as the permanent preservative agent because it does not become cloudy with sudden atmospheric changes, as is so frequently the case with methylated alcohol. The glass jar must be provided with a ground rim, upon which a glass cover of moderate thickness fits flush. The cover is luted to the bottle by a cement prepared according to the following formula :

Melt together, in an iron or earthenware vessel, one pound of gutta-percha and one pound of asphalt, and when they are thoroughly mingled pour them on a slate or stone slab which had previously been moistened with water. Roll the mass into cylinders of convenient length and thickness, and use the sticks in the same way as sealing-wax. The glass cover should be gently warmed before it is pressed upon the hot cement which has been spread along the rim of the glass jar. A weight, varying from four to twelve pounds according to the size of the jar, is placed upon the freshly-cemented top for a period of forty-eight hours, after which the superfluous cement is scraped away and a neatly applied ring of Brunswick black is painted round the edge and top of the cover in such a way as to conceal the line of junction.

The method here detailed is not applicable to every specimen. The hollow viscera, such as the uterus, bladder, etc., when they have been washed for twenty-four hours, and after the removal of redundant tissue, should be stuffed with tow, cotton-wool, or horse-hair, the aperture being subsequently sewn up. They should then be suspended for seventy-two hours in 64 per cent. methylated spirit, after which the stuffing should be removed, the preparation being placed in the ordinary mixture of methylated spirit and water, until they are ready for mounting. These preparations are put into strong spirit to cause them to set in the form given to them by the stuffing. Structures which deteriorate in water, such as the brain and other nervous tissues, and those in which it is desired to preserve, as far as possible, the original colour, should be placed at once in strong spirit.

Large anatomical and physiological preparations may be embedded in plaster of Paris, in white earthenware basins with ground rims, the basin being subsequently filled with diluted proof spirit, and the cover sealed down with a cement made by mixing together litharge and gold size to form a paste. As in the case of the glass jar, a weight must be placed upon the cover to keep it firmly adherent to the basin until the cement begins to dry, which will not usually take place much under a week.

Specimens showing colloid degeneration, tubercular synovitis, villous tumours, etc., may be preserved in a mixture of equal parts of glycerine and distilled water, to which has been added a trace of corrosive sublimate (1 part in 1500) or of pure carbolic acid.

In a few cases where it is necessary to preserve as far as possible the colour and translucency of the preparation, as in voluntary muscle infested

with trichinæ, Wickersheimer's fluid may be employed. This fluid can be obtained from the importers, 26, Finsbury Pavement, E.C., at the price of 2s. 6d. per quart bottle, or it may be manufactured according to the accompanying formula :

Alum	100 grammes.
Caustic potash	60 ,,
Chloride of soda	25 ,,
Nitrate of potash... ...	12 ,,
Arsenious acid	10 ,,

Dissolve the ingredients in three litres of boiling water. Cool. Filter, and afterwards add to each litre of fluid 400 cc. of glycerine and 100 cc. of proof spirit.

The fresh preparation should be entirely immersed in the fluid for ten days, and it should then be suspended in a glass jar with a small quantity of the fluid beneath it. The jar should be sealed in the usual manner. Neither the glycerine process nor Wickersheimer's fluid, however, have been found to yield very satisfactory results, and for valuable specimens it is better to adopt the alcohol method.

The glass jars should be round and of clear white glass free from bubbles. Large oval bottles frequently crack spontaneously, owing, so far as can be ascertained, to the extreme difficulty which is experienced in getting them properly annealed. The square bottles made in Holland, although very much cheaper and useful as stock bottles, are not of sufficiently good glass to be of much service in a well-kept museum. The covers of the basins, as well as of the large glass jars, should be provided with a hole plugged with a well-ground glass stopper—as, apart from the convenience of filling them without disturbing the cement, it will serve to prevent the covers cracking across as a result of variations in the bulk of the spirit due to alterations in the atmospheric conditions.

Small specimens, such as ovaries, sections of human eyes, etc., may be conveniently preserved and shown, by means of glycerine jelly, in the following manner :

After clearing away all redundant tissue, the specimen should be placed in Müller's fluid for a month. If its contents are suspected to be fluid, or semi-fluid, it should be frozen by placing it in a mixture of powdered ice and salt, and whilst it is hard it should be divided in the required direction with an ordinary sharp table-knife. The colouring matter of the Müller's fluid must next be dissolved out by placing the specimen in a solution of chloral hydrate (40 grains to the ounce), the chloral being changed as often as it becomes discoloured. The preparation is then placed for two or three days in a mixture consisting of one part of glycerine and three parts of water, from which it is transferred, for forty-eight hours, to a stronger mixture of equal parts of glycerine and water. It is now ready to mount in glycerine jelly, which is made thus : One part

of Coignet's (Paris) gold label gelatin* is soaked in six parts of distilled water until it is thoroughly swollen. It is then heated, and six parts of glycerine, to which a trace of pure carbolic acid has been previously added, is mixed with it, and the whole is filtered whilst it is hot through white filtering paper or cotton-wool, the heat being maintained during the filtration by means of a funnel with a hot-water jacket. The resulting jelly is preserved for use in a stoppered bottle.

To mount in glycerine jelly, small glass capsules similar to those employed in histological work are required, but they should be made of thicker and more brilliant glass. The jelly is liquefied by placing the bottle which contains it in hot water, and it is then poured over the preparation, which has been previously placed in the capsule. In the case of sections of the eye, care must be taken not to pour the jelly over the cut edge, lest the retina be detached. All bubbles should be carefully removed, and whilst the jelly is setting the specimen should, if necessary, be kept below the surface by gently pressing upon it with a needle fixed in a handle. When the jelly is quite firm its surface should be covered with an even coating of zinc paint, over which again a layer of shellac may be spread.

* Price 2s. 10d. per pound. The ordinary gelatine sold in packets in this country, even Nelson's No. 1 gelatin, used for photographic purposes, will not give a transparent jelly.

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