# JOURNAL

## OF THE

# ROYAL MICROSCOPICAL SOCIETY:

# CONTAINING ITS TRANSACTIONS AND PROCEEDINGS.

AND A SUMMARY OF CURRENT RESEARCHES RELATING TO

ZOOLOGY AND BOTANY

(principally Invertebrata and Cryptogamia),

MICROSCOPY, &c.

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FELLOWS OF THE SOCIETY.

## FOR THE YEAR

1895.



LONDON:

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OF MESSRS. WILLIAMS & NORGATE ; AND OF MESSRS. DULAU & CO.

# III.—A Portable Microscope by J. Zentmayer of Philadelphia.

## By Edward M. Nelson, F.R.M.S.

(Read 21st November, 1894.)

This instrument, while probably the smallest portable Microscope in existence, is nevertheless a thoroughly useful one for real practical work. It is not, therefore, what so many of these pocket Microscopes actually are, a toy. The box in which it is packed is unusually small, measuring  $4\frac{5}{8} \times 3\frac{1}{8} \times 1\frac{5}{8}$  in.

A glance at fig. 4 will show that a very early feature is retained by making the box the stand (J. Marshall's Microscope was mounted in this manner, 1704).

This Microscope has a firm and well-made coarse-adjustment. The stage,  $2\frac{5}{8} \times 1\frac{3}{8}$  in., is plain, having a wheel of diaphragms let into



its thickness; it now has a sliding bar, but originally it was fitted with spring clips, of the Powell type, fixed below the stage. One word about this sliding bar before passing on. Mr. Rousselet, the owner of the instrument, with his usual ingenuity, has fitted whalebone instead of steel springs below the lugs of the sliding bar. This is quite a novel feature in microscopical construction, and a smoother sliding bar I have never used. This is a point Microscope makers might adopt with advantage; a steel spring always works stiffly on a brass plate; if the spring is made of metal it ought to be brass also, but whalebone is better.

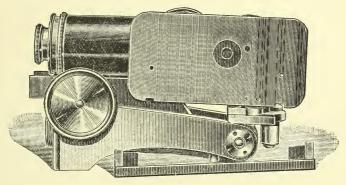
The body, which is  $2\frac{3}{4}$  in. long, is tapped with the Society's

screw, it has a draw-tube, which permits of a total extension of  $5\frac{1}{4}$  in.; it has one Huyghenian eye-piece, and by drawing this out another inch of tube-length can be obtained. The illuminating apparatus consists only of a concave mirror. The manner in which the stage is mounted

is peculiar: it, in common with those of most portable Microscopes, revolves on a pivot, but in this one the pivot is not in a line with the axis, but is a little towards the right-hand side of the stage; and curiously the hole in the stage is not in its centre, but is displaced 1/S in. nearer to the right-hand side. When the stage is rotated into position it is held there by a spring notch.

To pack up the instrument the legs are bent back against the limb; the stage, being released from the spring notch, is turned through 3/4 of a whole revolution, until it lies along the body as in fig. 5. The mirror, by means of its cranked arm, is turned against the other side of the body; the sliding arm however packs separately in the lid of the box.

FIG. 5.

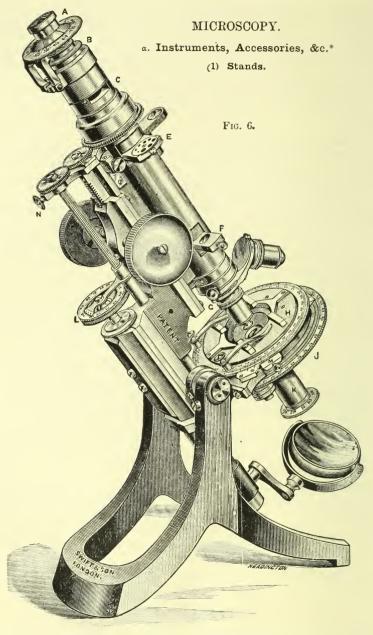


There is a new feature about the box, it has a stout brass plate attached to the bottom to impart steadiness. The weight of the box is 13 oz., and that of the Microscope with an objective is 17 oz. My Jubilee Microscope,\* with objective and substage condenser, weighs 14 oz., and its stand 2 lb.; its foot might screw into the lid of its box, and thus the stand might be dispensed with. The Zentmayer has the more rigid limb and legs, while mine has the more rigid stage. The size of the Zentmayer when closed, and without its box, is  $4\frac{1}{4} \times 2\frac{1}{5} \times 1\frac{3}{8}$  in., while that of the Jubilee is  $4\frac{5}{8} \times 3 \times 2$  in.; its body, which is 4 in. long, is separate. The distance of the optic axis from the limb in the Jubilee is  $1\frac{3}{16}$  in as against 3/4 in the Zentmayer. This is a defect in the Zentmayer, for when the sliding bar is on the stage nothing can be seen on a  $3 \times 1$  slip higher than 1/8 in. from the centre. If an object were higher than this the slide would have to be reversed. This might not make much difference beyond a slight inconvenience with mounted objects, but it would be very inconvenient whilst following living objects. If the sliding bar were replaced by the original spring clips, a little more but still an insufficient amount of play would be given to the slide.

\* See this Journal, 1887, p. 1013, figs. 238, 239.

To sum up the advantages and disadvantages of both models. The advantages in the Zentmayer form seem to me to be rigidity of limb and legs, and the body taking the Society's thread; while that of the Jubilee is the firmness and size of the stage, and the possession of a substage condenser. The disadvantages of the Zentmeyer are the insufficient distance between the optic axis and limb, and the want of a substage condenser; while those of the Jubilee are the weakness of the bar, the separation of the body in packing, and its not having the Society's screw.

In this summary I do not wish to make any invidious comparisons between Zentmayer's and my own model, but merely to point out their respective advantages and defects, so that any future designer of a portable Microscope may avoid the errors of both designs.



\* This subdivision contains (1) Stands; (2) Eye-pieces and Objectives; (3) Illuminating and other Apparatus; (4) Photomicrography; (5) Microscopical Optics and Manipulation; (6) Miscellaneous.

Messrs. Swift and Son's Improved Dick Petrological Microscope. -The original form of this stand was described by Mr. Allan B. Dick in this Journal in 1889, pp. 432-6, fig. 57. The present instrument (fig. 6) differs from the ordinary Dick model in having an independent revolving stage, which allows the object to be revolved in the field of the cross-wired eye-pieces; whereas in the first stand the object remained stationary, and the eye-piece revolved instead.

In the present stand an achromatic convergent system is provided, the upper lens of which slides in and out of the centre of the stage for use with high and low powers; being flush with the surface of the stage, it is always in contact with the object, and when an immersion fluid is used it gives an angle of 1.05. The lower lenses of the system are moved up or down by a revolving collar. The convergent system can easily be adapted as a spot-lens for any objective from 2 in. to 1/8 in.

Immediately below the system, and above the polarizer, is a small iris diaphragm. The polarizer is made to swing out of the axis of the Microscope to allow the achromatic condenser to be brought into use. The nose-piece is a self-centering one. The upper horizontal slide E has a revolving diaphragm of apertures for viewing rings and brushes in minute crystals.

Messrs. W. Watson and Son's "Grand Model" Van Heurck Microscope.—This instrument (fig. 7) is constructed on similar lines to Messrs. Watson's "B" Van Heurek Microscope figured in this Journal (1893, p. 92), but possesses the following special features :---The stage has rectangular mechanical movements, controlled by two stationary milled heads, working on one centre; 1 in. of motion is afforded to the stage plates in either direction. The whole stage can also be completely rotated in any position. The base-plate carrying the stage is continued in one casting round the sides of the 1 mb and fixed by screws, instead of being screwed to the front of the limb as in the ordinary way, and the bolt on which the instrument is inclined goes through the whole-limb and stage supports-thus imparting unusual firmness. In order to incorporate these alterations an increase of size of the Microscope has been necessary, and all the parts are more massive than in the original stand. The tripod foot is cork-shod, and has a spread of over 10 in. in each direction.

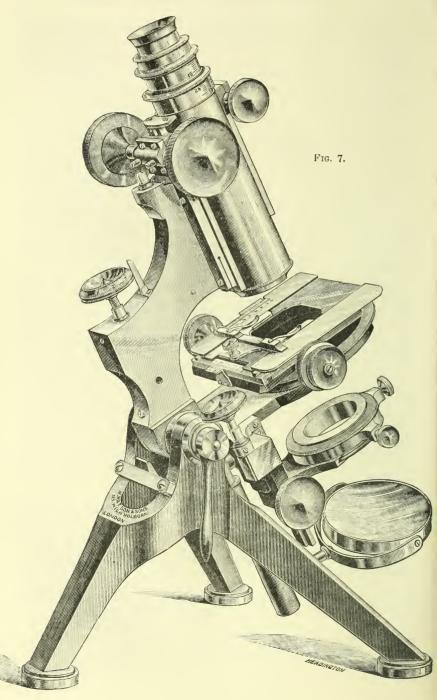
Zeiss new Mechanical Stage for Stand Ia.-For this stand Messrs. Carl Zeiss have recently constructed a mechanical stage which differs from that formerly supplied by them. The object in designing this new type of stage was to obtain, without diminishing its size and the exactness of its movements, an instrument of such solidity as to admit of its being permanently left on the Microscope and thus rendering the use of a separate stage superfluous.

The object-slide is placed in the usual manner with its shorter edge against the left stop A (fig. 8) and the left end of the lower long edge is pressed against the frame ledge R. The other stop B, which slides in a slot, is then placed against the other edge of the slide so as to hold it firmly. Stop A may by means of a screw h and a set pin be fixed at different points of the frame, there being a series of holes provided

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for the purpose, so as to adapt the object-holder to slides of various sizes.\*

This frame together with the stops and object-slide is moved laterally by means of the milled head K, fixed in a slanting position towards the

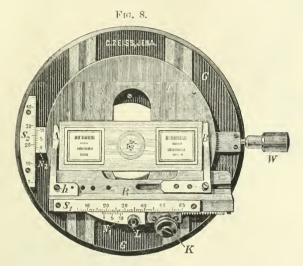
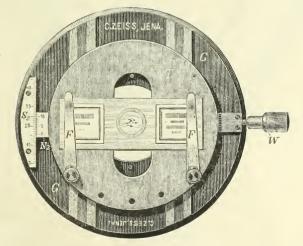


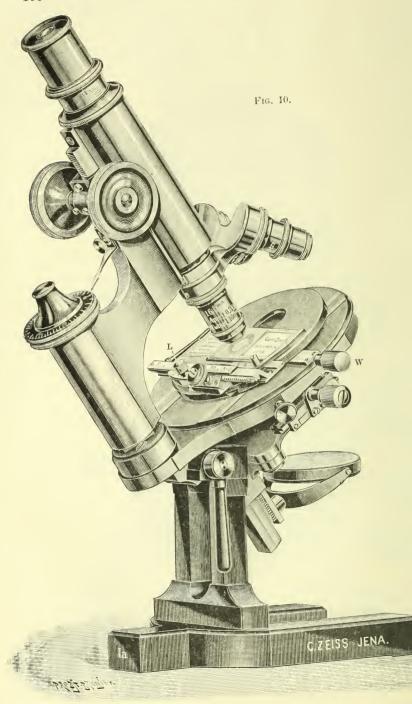
FIG. 9.



back and controlling a rack slantingly fixed to a lower side of the frame. The available lateral movement measures 50 mm. The guiding

\* Screw h is to be loosened by means of a screwdriver. Messrs. Zeiss have intentionally used an ordinary slit-head screw, since as a rule the position of the stop is adjusted once for all, and changes in the position of the stop would preclude the use of the scale as a finder.

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of the movement is effected by a dove-tail bar attached to a lower portion of the frame and is completely covered. The amount of the movement of the lateral position of the frame can be read by scale  $S_1$ and vernier  $N_1$ .

The milled head W projecting from the side of the stage is connected with a pinion, working a rack attached to the under side of the stage T on the right, and effects the forward and backward movement of the stage. The stage is made to move accurately and smoothly by means of two guide strips (not visible in the illustration). The four strips rising from the base shown in the figure are ground perfectly plane and smooth and serve as sliding surfaces for the guides. The magnitude of the movement is read off scale  $S_2$  by means of vernier  $N_2$ . The range of the movement in this direction is 35 mm. The milled head W also serves as a handle for rotating the entire stage.

The movable stage disc T is provided with an oval slot running in the direction of the movement. This slot is conically expanded downwards, and the base-plate G, which remains in a fixed position on the Microscope, has a circular opening in the centre. The arrangement admits of contact being established between the object-slide and the front surface of the condenser at any position of the stage. The makers state that all sensitive parts are well protected from dust and other influences. To set free the entire surface of the Microscope stage, unscrew the small vertical head L, and the whole frame R, which is fixed in position by two set-pins and held down by this screw, may be lifted off, and the stage then presents the appearance shown in fig. 9.

The stage is sufficiently large to take a culture plate or dish, or an object-slide of any desired form may be fixed by means of the springclips FF.

The stage is attached to the stand (fig. 10) and detached from it in precisely the same manner as in the case of the older stage.

#### (3) Illuminating and other Apparatus.

Differential Object-carrier.\*—Dr. H. E. Hildebrand has devised a new form of object-carrier for use in morphological work, where moving living objects are being studied. It allows, without changing the position of the guiding hand, both of constant tracks being kept, as in the ordinary mechanical stage, and also of any movements required by the object being given. This is effected by a difference in the friction between the surfaces for the back and front movement and those for the side to side movement. This difference in the friction can be ignored by the guiding hand in the production of any desired movement, but is allowed to control the motion when constant tracks are to be described.

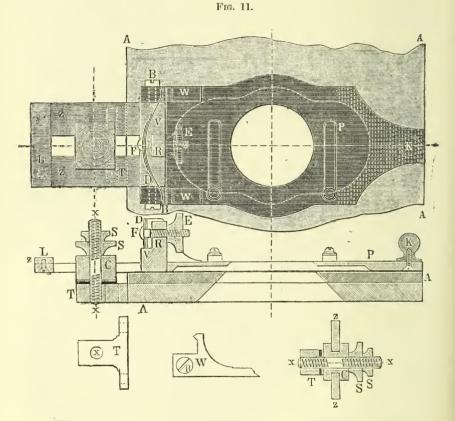
The object-carrier consists essentially of a plate with central aperture lying across the Microscope-stage, which is provided with a pressure spring, and by means of a grooved head can be rotated about a stationary vertical rod and also displaced laterally.

The steel rod x (Fig. 11), as the axis of all movements, is firmly fixed in its base-plate, the T-piece T, which is of such a length that

\* Zeitschr. f. wiss. Mikr., xi. (1894) pp. 304-12.

the pillar x stands in the middle of the slit of the plate Z, when the whole instrument lies upon the Microscope-stage in such a way that the apertures of the stage and the plate P are concentric.

The grooved head C is a rectangular block with a central smooth bore of such a size that it fits tightly on the rod x. Two of its opposite sides are provided with grooves in which the arms of the plate Z slide. The part of the rod x which projects above the head C, possesses a screwthread for two screws SS, the upper for clamping the lower. These serve for the exact adjustment in height of the whole instrument above the Microscope-stage.



The two-armed plate Z, which forms a long rectangle, consists of u single piece. On the length of its arms depends the extent of the lateral displacement of the object, which should amount to at least 3/4 in. After the introduction of the head C, the arms at the further side are closed by the cross-piece L which limits the displacement in this direction, while at the opposite side the plate is closed and is there also strengthened by a prismatic piece V, on which is a block R for the pressure spring D. The four-sided prism V fits between two projecting

pieces of the plate P, and is connected with the plate P by the screws BB.

On the spring D depends the proper functioning of the instrument. It is a steel spring in the shape of an arc, and is provided in the centre with a screw-bolt F which fits in a boring of the block R. The tension of the spring is regulated by the screw E. The pressure of the spring is transmitted to the plate P by means of the two angle-pieces WW, so that the plate is pressed down upon the Microscope-stage AA.

The plate P with all its accessories is cast in one piece, and has a large aperture for the complete use of the Abbe condenser. The dotted line in P marks the outline of the aperture on the under side of the plate. The whole plate should be made of aluminium to diminish the weight, so that a slight spring pressure would suffice in order to prevent a sliding of the carrier in an inclined position of the stand.

To prevent scraping in the sliding motion, about a third (the dotted part in the figure) of the under side of the plate P is covered with a thin woollen material. As regards the method of using the instrument, it is clear that the vertical rod x with the grooved head C always keeps the plate Z horizontal. It is otherwise with the plate P which is inclined by the pressure of the spring against the stage AA of the Microscope. This pressure, however, induces a pressure of the arms of the plate Z in the grooves of the head C, since here a variation from the horizontal plane is impossible. Since, further, the force of the spring acts on levers of unequal length—from the hinge BB to the rod x on the one side, and from the stage AA on the other—therefore the friction on the stage must be correspondingly smaller than that in the grooves of the head C.

In the practical use of the instrument, first the plate P is set parallel to the stage A of the stand by means of the two screws SS. The necessary tension is then given to the spring, which will vary according to the inclination of the Microscope.

By loosely holding the knob K between the thumb and first finger, the preparation on the carrier may be made to describe the same curve backwards and forwards. In this case the guiding hand makes no attempt to overcome the greater friction in the grooves, while the slight friction on the Microscope stage A is scarcely felt. A firmer pressure on the knob K is required to make the carrier move to and fro in the grooves C. By these two movements the whole preparation can be systematically examined. But if it is desired to follow the outline of an object or the course of a curve in a drawing, in this case, the hand holding the knob K must take no account of the resistance offered by the friction in the grooves C, but must follow the curves and outline of the object as they present themselves.

The figure represents the instrument in two-thirds of its natural size.

A new Drawing Apparatus.\* — Dr. S. Czapski states that the following conditions should be satisfied by a good drawing apparatus :—

(1) The light from the image must not to any great extent be weakened by the apparatus.

\* Zeitschr. f. wiss. Mikr., xi. (1894) pp. 289-98.

(2) The image of the drawing board must reach the eye with the least possible loss of intensity and coaxial with the microscopic image.

(3) There must be an arrangement by which the relation of the intensities of these two images can be changed within sufficiently wide limits; and this arrangement, as in Bernhard's apparatus,\* must allow of a change not only of the apparent brightness of the plane of the drawing but also of the intensity of the microscopic image.

(4) The apparatus must be adjustable in height and capable of being centered in its horizontal plane.

(5) It must be possible to easily separate the apparatus from the eye-piece and replace it again in its original position at will.

(6) The image of the plane of the drawing and the image of the microscopic object projected on it must be seen with the apparatus without any distortion.

The author describes the latest drawing apparatus offered by the firm of Zeiss, and shows to what extent it fulfils the above conditions.

As regards conditions 1 and 2, the author comes to the conclusion, as the result of numerous experiments which he has made, that the wellknown method of Schröder, Govi, and others, which consists in the use of a glass plate with a thin metallic deposit on its surface, does not sufficiently correspond to the requirements, since the light passing through the metallic layer is too much weakened. He accordingly adopts instead of this essentially the arrangement of the original Abbe camera, viz. two rectangular prisms with the hypothenuses cemented together, of which one is silvered with a small portion of the deposit in the centre scratched away, and with these a second mirror A (fig. 12) for transmitting the image of the plane of the drawing to this prism. But since one and the same prism, with a determined opening in its silver deposit, cannot suffice for all purposes and changes of magnification, an arrangement is added by which the prism P (fig. 13), with its fastening, can be easily taken out of the apparatus and replaced by another with an opening of different size.

With respect to condition 3, the author has sought to render the methods adopted by Abbe and Bernhard † less cumbrous by substituting for the discs, with their series of smoked glasses, an arrangement of two smoked glass wedges, after the principle of Babinet's quartz-wedge compensator, one wedge being made to move over the other, so as to form a plate of continuously varying thickness. By such an arrangement the problem in question meets with its complete solution, but for the apparatus in question the method was abandoned, since it raised the price of the instrument too considerably. Instead, a modified form of the Bernhard and Winkel arrangement was adopted. The smoked glass plates were set in the cylindrical wall of a small cap R (see figs. 12 and 13), which was simply placed over the prism. Each smoked glass can be in turn interposed in the path of the rays by turning the cap on its upper edge until a small pin engages in a corresponding small hole on the lower edge of the cylinder. In the cap are five smoked glasses of different strengths, while the sixth hole is left empty.

For diminishing the brightness of the image a disc B, as in Bernhard's apparatus, with four smoked glasses and a vacant space, is interposed between the prism and the eye-piece.

\* See this Journal, 1892, p. 263.

† Loc. cit.

The requirement of convenient adjustability in height is satisfied by the apparatus being attached to the body-tube by means of a clamping ring, while the adjustment from side to side is effected by the prism,

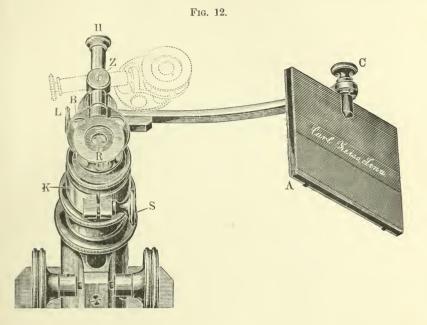
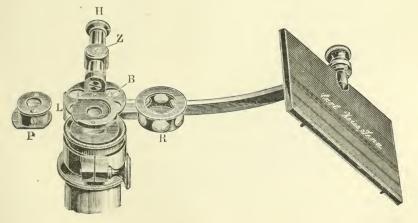


FIG. 13.



together with the cap and smoked glass disc, being centered from front to back by means of a screw H working through a spring socket, and from right to left by means of a second screw L, against which works a counter-spring not seen in the figure. SUMMARY OF CURRENT RESEARCHES RELATING TO

In order to pass conveniently from observation through the apparatus to observation through the free eye-piece, the prism, with its diaphragm arrangement, can be rotated to one side about a vertical pin Z. The return of the prism to the central position is marked by a spring-catch.

To obtain on the table an image of moderate size quite free from distortion, such an extent, and thereby weight, would have to be given to the side arm carrying the mirror, and to the mirror itself, as would be quite incompatible with the proper working of the apparatus. A moderate length, therefore, of 10.5 cm. was given to this arm, which was made of aluminium, while the size of the mirror was 7 to 8 cm. long and not much more than 5 cm. broad. To obtain drawings free from distortion, in combination with this apparatus a drawing table, similar to that described by Bernhard,\* must be used.

Improved Form of Bernhard's Drawing Desk.<sup>†</sup>—Dr. W. Bernhard has introduced the following changes in his desk for microscopical drawing which was described in this Journal for 1893, pp. 782–3.

(1) The drawing plate has, instead of the earlier swallow-tail groove, a very exactly worked brass groove on the upper plate, so that any shifting of the plate is prevented.

(2) The arrangements for determining the necessary height and inclination of the drawing board have been modified. The guiding arc on the left frame has now a division in  $5^{\circ}$ , while the edge of the right frame is provided with a centimetre scale, on which a pointer attached to the movable slide of the frame is adjusted.

(3) An adjustable rest for the drawing arm has been added to the instrument. This rest is connected with the drawing plate by a hinge, and is supported by a hinged piece, which is capable of extension, and has its lower end resting against the base-plate of the apparatus. By this arrangement the rest can be used in any position of the table, and can be folded down upon the drawing plate when the apparatus is not in use.

(4) For convenience in drawing an arrangement has been added by which the Microscope and drawing plate can be inclined towards the drawer. For this purpose the apparatus is connected by two hinges in front with a solid base-plate, so that the whole drawing table, with the Microscope, can be inclined towards the observer, and fixed at any inclination by a clamping screw.

A Silver on Glass Camera Lucida.<sup>+</sup>—Mr. W. Forgan gives a description of the various drawing apparatus which have been devised for the Microscope, and speaks of the difficulties experienced by many in their use. The method which he proposes is to place a small silver on glass mirror, such as is used as a *flat* in the Newtonian telescope, on the eyepiece of the horizontal Microscope, and so project the image down on the paper lying on the table.

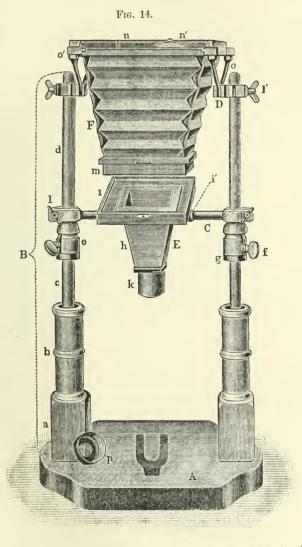
#### (4) Photomicrography.

Photomicrographic Apparatus.§—Prof. M. Lavdowsky's apparatus reminds one of that of Reichert, but differs from it in many respects,

- \* See this Journal, 1893, p. 782.
- † Zeitschr. f. wiss. Mikr., xi. (1894) pp. 289-301.
- 1 Proc. Scottish Micr. Soc., 1893-4, pp. 122-9.
- § Zeitschr. f. wiss. Mikr., xi. (1894) pp. 313-20.

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being much lighter and more portable. The total weight, inclusive of a large shutter, only amounts to 16 lb. On a moderately thick baseplate A (fig. 14) rise two wooden pillars B, which, by means of the two holders C and D, carry the two photographic cameras, the lower small



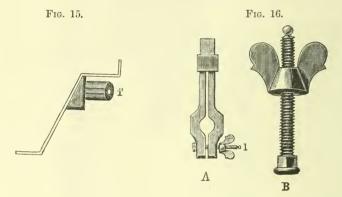
one E for small plates  $8 \times 8$ , and the upper one F, which fits into the lower, for larger plates,  $16 \times 18$ .

This division of the camera into two parts is very useful in working with petroleum light.

The base-plate A is 45 cm. long, 40 cm. broad, and 5 cm. deep, so that its dimensions are sufficient to adjust conveniently the Microscope, the petroleum lamp with the lens, and, if necessary, also the light-filter. The base-plate rests on a heavy round oak table, in which is a drawer for slides, &c. The under surface of the base-plate is thickly coated with felt.

The wooden pillars B consist of the following four parts: the base a, the movable intermediate piece b, and the two rods c and d, of which the first is screwed into the base a, while the second d is set on the upper end of the first.

The four-sided bases, which are 14 to 16 cm. high and 6 cm. broad and deep, are imbedded in the base-plate, and fixed by screws from underneath. Each has a screw-matrix of 3 cm. diameter, in which the rod c is screwed. The rotating intermediate piece b has the same matrix, and serves to fix the rod c. The rods are 2 cm. thick; the total length of the lower one is 38 cm., that of the upper 31 cm. The upper rods d are without screw-threads; they have at their lower ends the



blocks e, by means of which they fit over the lower rods; the rods are clamped together by the wooden screw f, which works through a slit made in the block. A metal ring q serves to strengthen the block.

The camera E consists of the following four parts:—the box E, 12 cm. high, lined with black cloth; the plate frame i of 16 cm. side-length and 10 cm. opening for light; the wide camera tube k, and the fork C which supports the camera.

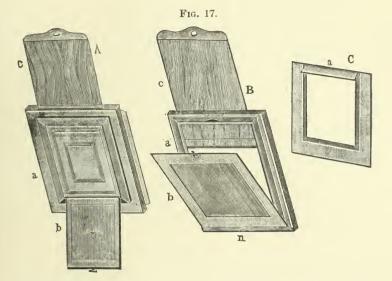
The connection between the upper and lower camera is made by a metal catch. The lower camera is attached to the stand in a peculiar way (fig. 14, I', and fig. 15). Beneath the frame on the side of the camera is a triangular piece of wood on which a projecting cylindrical socket, 3 cm. long, is screwed. In this socket fits a fork C, seen in fig. 16 A, which consists of a metal bar split down the greater part of its length, and having at one end a hole and a pressure screw (fig. 16 B) for fixing it on the rod.

When the lower camera is used alone, it is fastened by the screw l on the rod c, and the rod d is removed. The camera can be easily turned about the fork C, so as to be inclined in any position.

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The upper camera F consists of two wooden frames m and n, with a bellows between which can be drawn out to 20 cm. The upper frame is solid and is provided with two grooves and a catch n', which closes automatically after the slide has been introduced, and can be easily opened by pressure of the finger when the slide is to be taken out.

The upper part of the camera and its connection with the uprights is more complicated. By this connection the camera stands vertical, is movable on the rod d, and can be fixed in any position by the screw l'. The blocks D which fit over the uprights carry forks o, o', which support the corners of the frame.



Of the slides shown in fig. 17, A is for the lower camera, B for the upper.

The small slide consists of the frame a, with the cover b, and the slider c. The larger slide B is similar. Its size is  $16 \times 18$  cm., but it can be used for plates  $8 \times 8$ ,  $8 \times 10$ , &c., by inserting frames like that shown in C.

The First Photomicrogram in Natural Colours.\*—Dr. R. Neuhauss has made experiments in photography in natural colours by the methods of Valenta<sup>†</sup> and met with considerable success in the reproduction of mixed colours. An experiment was made in taking in natural colours a photomicrogram of a preparation of *Distomum lanceolatum* coloured bright red, with the internal organs black, yellowish brown, and dark red. The picture was taken with a linear magnification of 9 times, and with the use of the Auer incandescent light and Hartnack projection system on a bromsilver plate prepared by the method of Valenta. The plate was about ten thousand times less sensitive than those generally used for

- \* Zeitschr. f. wiss. Mikr., xi. (1894) pp. 329-31.
- † Valenta, E., 'Die Photographie in natürlichen Farben,' Halle a. S., 1894.

photomicrographic work, so that an exposure of three hours had to be given.

When developed the plate showed a very satisfactory reproduction of the colours of the original.

Simple Method of taking Photomicrographs of Opaque Objects.\* —Drs. E. W. Carlier and G. Mann have sought to obtain photomicrographs of the surface instead of sections of various animal and vegetable tissues.

The authors give a detailed description of the apparatus and process which they employed.

A horizontal camera made by Mr. Forgan, of Edinburgh, fitted to a large Zeiss Microscope, was used. The preparation was illuminated with a beam of light concentrated by a bull's-eye condenser. The light of an argand burner was found to be sufficient in most cases for focusing on the ground-glass screen. Magnesium ribbon fed through a piece of brass tubing was used for taking the photograph.

Ilford ordinary medium isochromatic plates were used. For timing the exposure an ordinary metronome set to half seconds was found to be very convenient.

As developer the old Ilford hydrokinone formula was used. After developing, the plates were immersed in an acetic acid bath, as by this process the negatives were rendered much clearer.

The best results were obtained with most objects when the incident light formed with the plane of the stage an angle of about  $40^{\circ}$ . Only low-power lenses such as No. 1 and 3 of Leitz were used.

Ĝelatino-chloride papers of the kind termed "Solie" (Eastman) were used for printing, and Eastman's combination toning and fixing bath for toning.

#### (5) Microscopical, Optics and Manipulation.

On the Passage of Light through a System of Spherical Lenses. -Mr. C. V. L. Charlier considers that the theory of the spherical aberration of light, although of the utmost importance in the construction of optical instruments, has not by any means been sufficiently developed to answer the requirements of practical optics. As contributions to the subject, the author intends to publish investigations:—

(1) On the image of a point by the passage of light through any number of centric lenses.

(2) On the choice of the radii of curvature, refractive indices, thickness and distances apart of the lenses, in order to obtain the best possible images.

(3) On the photographic images of stars.

As a preliminary to these investigations, in the present paper he gives the mathematical determination of the equation for the *aberration* curve as defined below.

A system of lenses is considered with refracting surfaces spherical, and with centres on one straight line, the axis of the system. Through the centre of curvature of the first of these surfaces a plane, called the

\* Proc. Scottish Micr. Soc, 1893-4, pp. 115-21.

+ Nova Acta Sci. Upsala, xvi. (1893) viii.

fundamental plane, is drawn at right angles to the axis. In this plane a circle of any given radius  $\kappa$  and with its centre on the axis is drawn.

If rays from a given point be considered before refraction as passing through this circle, after refraction they will form on any plane at right angles to the axis a curve. This curve the author calls the *aberration* curve for the radius  $\kappa$ .

With respect to this curve the author has demonstrated the following properties :---

It is an algebraic curve of the fourth degree. The coordinates can be represented by trigonometrical functions thus:

$$y = (\mu_0 + \mu_1 \sin \phi) \cos \phi$$
  
$$z = \lambda_0 + \lambda_1 \cos \phi + \lambda_2 \cos^2 \phi$$

where  $\phi$  is an angle in the fundamental plane between the y axis and the radius vector to the incident ray; and the five magnitudes  $\lambda$  and  $\mu$  depend on the constants of the system of lenses and on the position of the plane of the image.

The Secret of the Brownian Movement.\*-Mr. R. Meade Bache gives an account of the experiments which he has made in order to determine the cause of the Brownian movements. Robert Brown found as the result of a series of experiments which he made on finely crushed glass, various minerals, and many organic substances, that extremely minute particles of solid matter, whether organic or inorganic, when suspended in water, exhibit motions resembling in their irregularity the less rapid motions of some of the simplest animalcules of infusions, and states his belief that the motions "neither arose from currents in the fluid containing them, nor depended on the intestine motion which may be supposed to accompany its evaporation." More recently Herren Wiener, Exner, and Schultze have investigated these movements. Wiener concluded from his experiments that they have for their basis the movements which, by virtue of their molecular constitution, belong to fluids. Exner considered that the liveliness of the movement was heightened by light and heat.

The author, in his experiments, made use of finely divided carmine suspended in water. He found that no effect was produced upon the movement by the passage of a galvanic current through the liquid, by placing the liquid in the lines of force of a permanent magnet, nor by the application of heat and cold. Herr Wiener had been inclined to attribute the movement to the action of the red wave of light, but the author could detect no alteration in the movements when either a violet or a red glass was interposed between the source of light and the particles.

By observation for weeks, both with water-immersion lenses and also with a 1/15 dry lens, of liquid enclosed in a hermetically sealed cell the author could find no alteration in the movement, and concludes that evaporation has nothing to do with it. As the result of all his experiments the author therefore concludes "that it is not the particles which are moved by their own energy, or moved by any energy directly imparted to them from outside sources, but that it is the fluid that moves them."

\* Proc. Amer. Phil. Soc., xxxiii. (1894) pp. 163-77.

According to the author, in alcohol and in fixed and volatile oils the Brownian movement is not observable; it is a property of water and of water alone, and is caused by the mutual repulsion of the molecules of this liquid.

Mechanics and Optics at the World's Fair at Chicago, 1893.\*-Herr B. Pensky and Prof. A. Westphal give an account of the various exhibits connected with Mechanics and Optics at the Chicago Exhibition of 1893. In these departments the German firms were well to the fore. Polished plates of the various kinds of optical glass manufactured at the Jena glass-works were exhibited by the firm of Schott and Genossen. These included crown glasses, heavy and very heavy baryte-crown with high refraction, crown glass with low colour dispersion, phosphate and borate glass free from silica, ordinary flint glasses, Jena normal glass for thermometers, combustion tubing, &c. The firm of Carl Zeiss was well represented by a collection of their apparatus arranged in three divisions. The first division contained microscopical apparatus, viz. apochromatic and achromatic objectives, compensation eye-pieces, apparatus for examining objectives, projection apparatus, photomicrographic apparatus, Microscopes of all kinds for biological, crystallographic, petrographical, purposes. In the second division were the photographic lenses, in-cluding the well-known Zeiss Anastigmatics. The third division showed the productions of the firm in optical apparatus intended for physical and technical purposes, and included refractometers, contact-micrometers, spherometers, focometers, &c., made after the designs of Prof. Abbe. In the department of photographic objectives the firms of Steinheil, Voigtländer, and Schulze and Bartels had extensive exhibits; the first-named firm showed their well-known antiplanatics and their new apparatus for telephotography; the firm of Voigtländer exhibited a number of euryscopics and anastigmatics; while Schulze and Bartels were represented by the teleobjective of Dr. Miette and by a collection of telescopes and optical glasses of all kinds.

In the department of microscopy the firm of Voigt and Hochgesang was prominent with Klein's Microscope for mineralogical and petrographical purposes, and with Lehmann's crystallization Microscope and its accessories. The old Berlin firm of Schieck exhibited Microscopes for investigations on trichinosis, for entomological and other purposes.

Important exhibits of spectral, polarization, and photometric apparatus were made by the firms of Krüss, and Schmidt and Haensch. The first of these firms is responsible for the introduction of the Hefner lamp, and the latter for that of the Lummer-Brodhun apparatus in photometry. In the mineralogical division of the University Exhibition in the German section were exhibited petrographical Microscopes by Fuess and by Voigt and Hochgesang, Klein's Microscope for more exact mineralogical-petrographical investigations, Klein's heating-Microscope, Nörremberg's polarization-instrument, a collection of Fuess's mineralogical instruments, the crystal refractometer of Abbe, the total-reflectometer of Kohlrausch, and many other apparatus.

England was very poorly represented at the Exhibition. Ross and Co. exhibited Microscopes and a number of photographic objectives in

\* Zeitschr. f. Instrumentenk, xiv. (1894) pp. 133-6, 176-80, 210-14, 252-5, 327-31, 366-9.

which the Jena glasses were extensively used; the newest of these was the "Concentric" objective for landscape and architectural photography, consisting of two symmetrical double-lenses. Watson and Son, besides astronomical and measuring instruments, exhibited photographic and projection apparatus and Microscopes. Amongst the latter was shown the Van Heurck Microscope for work with high magnification, one of the peculiarities of which is the special draw-tube which allows of the use of the German as well as the English tube-length, according as the objective to be used is corrected for one or the other. The firm of Beck also exhibited a collection of Microscopes.

Other European nations were well represented in the department of Microscopy by the firms of Nachet, Reichert, and Koristka.

Amongst American exhibits the Gundlach Optical Co. showed their photographic objectives and Microscopes; and the Bausch and Lomb Optical Co. a large collection of their Microscopes, microtomes, and photomicrographic apparatus. A peculiarity in the Microscopes of the latter firm was a hemispherical diaphragm intended to replace the Zeiss iris-diaphragm.

#### β. Technique.\*

Practical Methods in Microscopy.<sup>†</sup>—This is an elementary manual of microscopical manipulation intended to guide such as have little or no previous acquaintance with the instrument, or the methods of examination and preparation of objects. It deals firstly very briefly, but perhaps sufficiently for an absolute beginner, with the phenomena of refraction, the formation of optical images, aberration, and so forth, followed by a description of the compound Microscope and its chief accessories, drawing instruments, microtomes, &c., polarized light having a short chapter to itself. Manipulation and the examination, preparation and mounting of specimens necessarily take up the major part of the work, and the pages devoted to making sections of vegetable and animal tissues, rock sections without a lapidary's wheel, and the cultivation and staining of bacteria, are up to date and quite as full and exact as is required by the class of student for whom the book was specially written. No mention, however, is made of the freezing process, which is rather an oversight considering the great convenience of such instruments as the Cathcart, and particularly Jung's automatic ether microtome, to those who do not devote themselves almost entirely to section-cutting, as, after all, the complicated celloidin and paraffin processes are only requisite for serial sections, or where the material necessitates continual internal and external support during the whole time it is under treatment. It is perhaps superfluous to state that the now indispensable chapter on photomicrography is not forgotten. The work is illustrated with the usual figures, and also by a series of process plates from photographs done with a low power. It is well printed, and commendably free from errors, typographical or otherwise, but in the examination of human blood (p. 79) the white corpuscles are

<sup>\*</sup> This subdivision contains (1) Collecting Objects, including Culture Processes; (2) Preparing Objects; (3) Cutting, including Imbedding and Microtomes; (4) Staining and Injecting; (5) Mounting, including slides, preservative fluids, &c.; (6) Miscellaneous.

<sup>†</sup> By Chas. H. Clarke, A.M., Boston, U.S., and London, 1894, 211 pp. 1895 . I

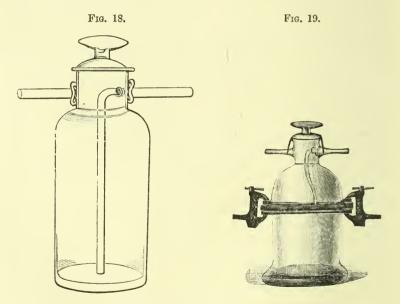
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stated to be smaller than the red, and plate IX., given as human liver, represents indifferently the cortical portion of the kidney.

A BEL, R.—Taschenbuch für den bakteriologischen Praktikanten, enthaltend die wichtigsten technischen Detailvorschriften zur bakteriologischen Laboratoriumsarbeit. (Handbook for Bacteriologists, containing the most important technical details for the work of the Bacteriological Laboratory.) 3rd edition of Bernheim's Taschenbuch. Würzburg, 1894, large 16mo, vii. and 56 pp.

#### (1) Collecting Objects, including Culture Processes.

Cultivation of Rhizobes.<sup>\*</sup>—Mr. A. Schneider cultivated rhizobes in the following way:—Instead of ordinary pepton gelatin he used a cultivation medium of agar made up with a watery extract of *Melilotus alba*. To the extract pepton, pancreatin, and NaCl were added in different quantities. The reaction of the medium was acid. The bacteroids of *M. alba* were artificially cultivated by incising a root-tubercle and then inoculating the medium from the wound. After 4–5 days white colonies of *Rhizobium Frankii* var. *majus* were formed. Most of them were mobile, and they multiplied both by spores and fission. The mobility was greatest in fluid media, and the character of the movements rendered the presence of cilia probable. By staining with Hoffmann's violet a cilium at both ends was demonstrated (sometimes two). Spores just developing had 3–4 cilia.



Apparatus for Cultivating Anaerobes.<sup>†</sup>—Prof. F. G. Novy describes a bottle for cultivating anaerobes which will hold four or more Petri's

\* Bull. Univ. Illinois, 1893, pp. 301–19, 3 pls. See Centralbl. f. Bakt. u. Parasitenk., xvi. (1894) pp. 631–5.

† Centralbl. f. Bakteriol. u. Parasitenk., xvi. (1894) pp. 566-71.

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capsules at the same time, and which can be used for working with hydrogen, carbonic acid or coal gas, or with pyrogallic acid. In shape it is somewhat like an exsiccator, and its neck and stopper are shown in fig. 19. The body of the bottle is in two moieties, which are fitted together very accurately, and joined by carefully ground flanges. The diameter of the body of the bottle (fig. 18) is 12 cm. and its depth is 12 cm. The stopper has a diameter of 2–3 cm., and by giving it a quarter turn, or 90°, the apparatus is closed. After putting in the material the two parts, the edges of which have been previously smeared with a mixture of beeswax and olive oil, are fitted together, and the chink covered with a broad rubber band. This is securely held by an iron clamp, tightened up by means of screws. The stopper must be prevented from jumping out by securing it to the neck with a rubber band.

Apparatus for Collecting Samples of Water.\*—Dr.C. Gonçalves has devised a very simple and inexpensive apparatus for collecting samples of water from any depth for bacteriological examination. The apparatus consists of a glass bottle supported on a metal plate weighing 2 kilos, and kept in position by a ring round the neck, and joined to two iron rods rising vertically from the iron plate by means of screws. Above the bottle the iron rods are joined by a cross piece placed at such a distance above the stopper that the latter can only be just raised, and not completely removed. From the stopper runs a wire, passing through a hole in the cross piece to the surface. Another wire, connected with another terminal cross-piece, supports the apparatus. A sample is obtained by just letting down the apparatus to the required depth, and then pulling the wire connected with the stopper, which returns to its place when the wire is released merely by its own weight.

Examining Water containing the Bacillus of Typhoid Fever.†— M. Grimbert made experiments for the purpose of ascertaining if it were possible to isolate the bacillus of typhoid fever from water also containing B. coli commune. For this purpose flasks containing sterilized water were inoculated with 1 ccm. of a typhoid culture and also with 1 ccm. of a coli culture. Neither in the flasks to which carbolic acid had been added nor in those without this addition could living typhoid germs be found after 48 hours. When the author inoculated 1 litre of sterile water with 1 ccm. of a typhoid culture, and with only 2 drops of a coli culture, and made gelatin plates after 3 days, only colonies of B. coli com. grew up. As the typhoid bacilli, when alone in the water, were still alive after the same period, it seems to follow that their disappearance was due to the influence of B. coli commune.

Cow's Milk and Cholera.<sup>‡</sup>—According to Dr. W. Hesse fresh cow's milk, so far from being a cultivation medium for cholera bacilli, actually kills these germs. The destructive process begins directly the bacilli are placed in the milk, and it is over in 12 hours at a temperature of  $15^{\circ}-20^{\circ}$ , or in 6–8 hours at incubation temperature. This destruction is independent of the acids in the milk or of the germs and their metabolic

<sup>\*</sup> Rio de Janeiro (Leuzinger et Filhos), 1893. See Centralbl. f. Bakteriol. u. Parasitenk., xvi. (1894) p. 257.
† La Semaine Méd., 1894, p. 230. See Centralbl. f. Bakteriol. u. Parasitenk.,

<sup>†</sup> La Semaine Méd., 1894, p. 230. See Centralbl. f. Bakteriol. u. Parasitenk., xvi. (1894) p. 586.

<sup>&</sup>lt;sup>‡</sup> Zeitschr. f. Hygiene u. Infektionskr., 1894, p. 238. See Centralbl. f. Bakteriol. u. Parasitenk., xvi. (1894) pp. 202-4.

products, but is an expression of the vitality of the living milk, and is instantly extinguished when milk is heated to  $100^{\circ}$  C. Exposure to the action of steam for 3 hours or more does not affect the germicidal power of milk, and this is to be ascribed to the increased acidity resulting from the action of steam. But an exposure to steam for less than  $1\frac{1}{2}$  hours renders milk a good cultivation medium. In a few days' time a reversal ensues, and this is probably the result of the growth of the bacilli, the formation of acid, and the coagulation of the casein. Yet even in acid and clotted milk viable cholera bacilli will be found at the expiration of a week. The practical outcome of these observations is that raw cow's milk is less suspicious than boiled as regards cholera bacilli.

Effect of Bile, Urea, and Borax on Cholera Bacilli.\*-Herren H. Leo and R. Sondermann have studied the effect of bile, urea, and borax on the growth of cholera bacilli. Fresh ox-gall was discontinuously sterilized by heating it for several days to 60° C. The urea and borax were dissolved in water and then steam sterilized. When the medium (gelatin) contained 50 per cent. of bile, its influence was favourable to the growth of the comma bacilli; if considerably more it became unfavourable but never fatal. This promotion of growth was probably due to the increased alkalinity imparted to the medium through the bile. though increased fluidity probably had some influence, as experiments made by adding water showed. These two factors, increased alkalinity and fluidity, overcame the inhibitory effect of the bile acids. Hence in the organism the bile can have no inhibitory effect, as the intestine rarely contains 50 per cent. The addition of 1.45 per cent. of urea had an evidently restraining effect, and this increased with increase of the amount of urea, though more than 4.5 per cent. could not be added without affecting the solidification of the gelatin. A 10 per cent. solution of urea was found to kill cholera bacilli in 20 hours.

With regard to borax it was found that 1:1000 exerted an inhibitory action, but none was observable when the proportions were 1:500<sup>0</sup>. A half per cent. borax solution possessed bactericidal powers, while a 5 per cent. solution killed cholera bacilli in 17 hours.

New Diagnostic Criterion of the Bacillus of Typhoid Fever.<sup>†</sup>— Though Bacillus typhosus and Bacterium coli commune may usually be distinguished by their behaviour in sterile milk, in saccharated media, and in pepton water, a further criterion is sometimes desirable. This, says Sig. C. Gorini, may be found by adding 2 per cent. of urea to a gelatin cultivation of typhoid. On the first two days the organism grows in the usual way, but on the third or fourth the gelatin, which was rendered cloudy by the urea, clears up, and fine white granules, apparently crystals of carbonate of ammonium, which are disseminated throughout very regularly, appear in the medium. Bacterium coli also forms crystals, but these only accumulate in little heaps along the inoculation track. Besides this numerous gas-bubbles can be seen, and these are apparently due to the splitting up of the urea into carbonic acid and ammonia.

\* Zeitschr. f. Hygiene, xvi. p. 505. See Centralbl. f. Bakteriol. u. Parasitenk., xvi. (1894) pp. 198-9.

† Giornale d. Reale Soc. Ital. d'Igiene, 1894, No. 7. See Centralbl. f. Bakteriol. u. Parasitenk., xvi. (1894) p. 713.

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Examining purulent Exudations for Bacteria.\*-Dr. Heim adopts the following procedure for examining exudations, e.g. of the pleural sac, and especially for streptococci. Before proceeding to puncture, the contents of two glycerin-agar tubes are poured into the capsules and allowed to set. The exudation having been removed with proper precautions, some 1/2 ccm. is injected into the peritoneal sac of a mouse, and 1-2 ccm. into that of another. Stroke cultivations are next made on the agar plates, and these are incubated with the lid downwards. The rest of the exudation serves to make microscopical preparations. Should there be a suspicion of tuberculosis, 1 ccm. should be injected into the peritoneal sac of a guinea-pig, and this should always be done if the exudation be of a serous character. If capsule cocci be present the mice will die within 24 hours, and then microscopical preparations are easily made. The colonies of capsule cocci on agar plates are closer and less diffused than those of streptococci. The microscopical preparations in these cases show the lancet-shaped diplococci or chains of cocci. White mice are the animals most sensitive to streptococci, and when injected subcutaneously at the root of the tail die in from 2-7 days, and the cocci are demonstrable in almost every part and organ.

Sterilizable Injection Syringe.<sup>†</sup>—Prof. F. Loeffler recommends for experimental injections a syringe which can be sterilized by steam or alcohol-ether and which works satisfactorily. The piston-rod and its plunger are made of metal. The latter is a disc of thin metal with a sharp edge, and its diameter is such that the piston can be moved up and down without touching the inside of the syringe. Over the disc is stretched a thin rubber cap, which is fastened behind by silk thread or fine iron wire. When thus fitted and lubricated with vaseline or even water it glides up and down the canula quite easily if the proper thickness of rubber have been chosen. There is no escape of fluid behind the plunger. Syringes of this construction may be made to hold 1-50 ccm. of fluid. In order to ensure that the syringe works perfectly it is necessary that the internal diameter of the canula should be quite regular throughout, and that the plunger should not be too thick.

Filtration of Agar-agar.<sup>‡</sup>—The ordinary methods of filtering taking too much time, Dr. W. St. C. Symmers employs the method used at the Pasteur Institute. The important requisite in this method is the filterpaper known as the "papier chardin," made by Cogit et Cie. The agar-agar is heated in an autoclave to 120° C., and poured at once on to the filter-paper in a cold funnel. It filters as rapidly as nutrient gelatin does in the ordinary method, and a litre may be obtained in half an hour.

- HÜPPE, F., & A. FAJANS-Ueber Kulturen im Hühnerei und über Anaërobiose der Cholerabakterien. (On Cultures in Fowls' Eggs, and on the Anaerobiosis of Cholera-bacteria.) Arch. f. Hygiene, XX. (1894) pp. 372-83.
- KLEIBER, A.-Qualitative und quantitative bakteriologische Untersuchungen des Zürichseewassers. (Qualitative and Quantitative Investigations on the Bac-teriology of the Water of the Lake of Zurich.)

Zurich-Oberstrass, 1894, large 8vo, 57 pp., 1 fig., 1 pl.

\* Münchener Med. Wochenschr., 1894, No. 22. See Centralbl. f. Bakteriol. u. Parasitenk., xvi. (1894) pp. 799-801. † Centralbl. f. Bakteriol. u. Parasitenk., xvi. (1894) pp. 729-31.

1 Brit. Med. Journ., No. 1765 (1894) p. 951.

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LEMIÈRE, G.-Un appareil simplifié pour la numération des bactéries. (A simplified Apparatus for Counting Bacteria.)

Journ. Sci. Méd. Lille, 1894, pp. 169-75. UNNA, P. G.--Natürliche Reinkulturen der Oberhautpilze. (Natural Pure Cultures of Epidermal Fungi.) Mtsh. f. Prakt. Dermatol., 1894, pp. 257-67.

#### (2) Preparing Objects.

Centrosomata.\*—Dr. M. Heidenhain observed the following technique in his researches on centrosomata. For preserving he used especially sublimate and Flemming's acid mixture; for staining, Bordeaux R, Biondi's solution, iron-hæmatoxylin, triacid-solutions of Ehrlich. The best nuclear staining he has yet seen was got with thionin (Ehrlich-Hoyer). For differential staining he used, in the first place, Bordeaux K, anilin-blue, or methyl-eosin; but chiefly the first, then the iron-hæmatoxylin. The chapter on methods is well worth the attention of histologists.

Hardening of Chick's Egg in toto.<sup>†</sup>—As it is almost impossible to take out the contents of a fresh egg entire after the first week of incubation, Mr. S. Hirota recommends that the egg should be patiently tapped until the greater part of the shell is broken into small pieces; these should then be separately removed, and the underlying shell-membranes left intact.

If the egg has been in incubation less than two weeks, care should be taken not to injure the large blood-vessels of the allantois; if they are injured, the blood should be coagulated by blowing at the point. At this stage, as the contents are still soft, a large piece of shell should be left to support the envelopes in their relative positions till they have become hardened in Kleinenberg's fluid. Next day the fluid should be pipetted off and carefully replaced by alcohol.

When the egg has been in incubation for more than two weeks' there is no danger of injuring the blood-vessels, even the inner shell-membranecan be removed without fear of bleeding if the whole be put in Kleinenberg's fluid for half an hour or even less. When the contents are entirely cleared from the non-cellular envelopes the specimen is put in the same fluid for half a day; the fluid, when it has done its work, can easily be replaced by alcohol.

In both cases yolk, albumen, and amniotic fluid should be removed, as these substances coagulate in alcohol, and cannot then be removed.

Examination of Pedal Gland of Pulmonates.<sup>‡</sup>—M. E. André used the methods of teasing when fresh, and teasing after maceration in various reagents as well as that of sections. As a macerating reagent he found 1 per cent. osmic acid and 1 per cent. bichromate of potash to be good, if used alternately for from two to seven days, when studying the glandular cells; for the epithelial cells of the excretory canal saturated solutions of boric and salicylic acids kept at a temperature of from 25° to 30° for two or three hours, and a 3 per cent. solution of chloral hydrate were found to be good, especially if a suitable nuclear stain was added.

<sup>\*</sup> Arch. f. Mikr. Anat., xliii. (1894) pp. 423-758 (7 pls.).

<sup>+</sup> Journ. Coll. Sci. Imp. Univ. Japan, vi. (1894) pp. 367-9.

<sup>‡</sup> Rev. Suisse Zool., ii. (1894) pp. 293-5.

Examination of Hirudo medicinalis.\*-Dr. J. M. Croockewit killed his leeches with 96 per cent. alcohol, and put them into alcohol, to which was added picric acid to dissolve the carbonate of lime in the teeth, for a day; the best staining results were got with Böhmer's hæmatoxylin.

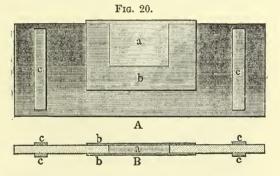
Mitosis in Ova of Ascaris.†-Herr V. Herla found the best fixative to be a mixture of 1 part of glacial acetic acid with 5 parts of absolute alcohol. His stain was a mixture of the following (parts by weight):-Vesuvin ·25, malachite-green ·25, distilled water 100, glycerin 10.

Division of Amœba.t-Dr. F. Schaudinn fixed Amæbæ with hot concentrated sublimate, or in platinum chloride-osmo-acetic acid (Herrmann), or in Kleinenberg's picro-sulphuric acid. He washed them in the first instance with 63 per cent. iodine-alcohol, in the second with water, in the third with 63 per cent. alcohol. Those fixed with sublimate were best stained with iron-alum and hæmatoxylin (Benda-Heidenhain), but Ehrlich's hæmatoxylin, &c. yield good results.

#### (3) Cutting, including Imbedding and Microtomes.

Micro-Aquarium which can also be used for Paraffin Imbedding.§ -Dr. F. Schaudinn has devised a small cover-glass aquarium to replace the Cori stage-aquarium when small organisms not visible with the unaided eye are to be examined.

In an ordinary slide is cut a rectangular opening a which reaches about up to the middle of the slide. On each side of this, cover-glasses



b are cemented by Canada balsam, as seen in the figure (fig. 20). On both sides of the cover-glasses small strips of glass c are cemented. In this way a small aquarium is obtained into which water and creatures can be introduced by means of a pipette. The slide can be used in the horizontal position, since owing to capillarity no water can flow out.

For keeping the water fresh, green algæ can be used or fresh water can be introduced by means of a woollen thread.

For the observation of living organisms the aquarium offers the advantage that even with high magnification the whole space can be easily examined.

Organisms which have fastened themselves on the walls of the cover-

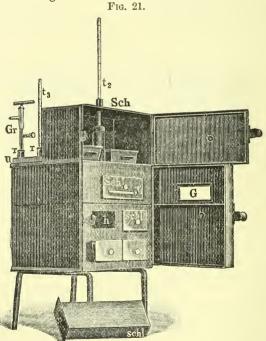
- \* Tijdschr. Nederl. Dierk. Vereen, iv. (1894) pp. 297 and 8.
- † Arch. Biol., xiii. (1893, published 1894) pp. 423-50 (5 pls.).
   ‡ SB. K. Preuss. Akad., 1894, pp. 1029-36 (5 figs.).

  - § Zeitschr. f. wiss. Mikr., xi. (1894) pp. 326-9.

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glass can be killed in this state, and after removal of the water preserving liquid can be introduced. The colouring and hardening of creatures so fixed is very convenient. The author has also used the aquarium for the imbedding of small objects in paraffin. For Amæbæ and similar organisms, for example, a triangular piece is cut out of the slide, and the cover-glasses are cemented on with isinglass. The creatures introduced sink to the bottom into the point of the triangle. The xylol in which they were preserved is removed and replaced by paraffin. The slide is then placed in cold water, when the paraffin solidifies and separates from the walls of the aquarium. The isinglass at the same time dissolves in the water, and the paraffin-block thus set free is ready for cutting.

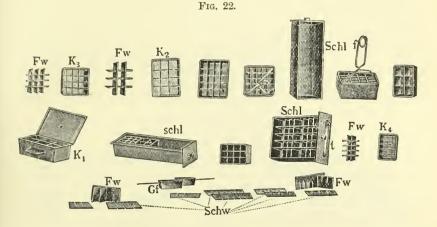
The aquarium can also be used for orienting small objects for cutting. For this purpose some of the very finely fibrous material sold under the name of Penghawar-Djambie is introduced with the xylol into the aquarium. A small round hole is made in this with a rounded wooden rod, and the object to be oriented is placed in it. The object is held in any desired position by means of the fine fibres and can be readily adjusted under the Microscope. The xylol can then be replaced by paraffin, or the orientation can be originally made in paraffin on the hot stage.



Apparatus for Paraffin Imbedding.\*—Dr. A. Kolossow describes an apparatus for imbedding objects in paraffin, which though much

\* Zeitschr. f. wiss. Mikr., xi. (1894) pp. 154-61 (5 figs.).

dearer than the Naples water-bath, is more suitable for paraffin imbedding. The apparatus consists of a quadrangular box of copper (fig. 21), tinned on its inner surface. It is about 24 cm. broad and 26 cm. high, and it is divided by a horizontal partition into two compartments, the upper being 10 cm., the lower 16 cm. high. In the upper one and in the lower are four spaces for the reception of drawers made of nickeled copper. The drawer-cases are supported by upright pieces. 'On the top of the front of the box is a cupboard, behind which is a free space of 10 cm. To the bottom of the cupboard and to the top of the box a sort of damper, in which is a double row of holes 15 cm. in diameter, is fitted.



At the top left corner are two openings, both of which communicate with the lower department, and through which it can be filled with water. In one is fixed a thermometer  $t_3$  and in the other a gas-regulator. The level of the water is marked by a tube. At the bottom of the box is a tap for letting off the water. The thermometer  $t_2$ , the end of which dips into a glass vessel filled with water, indicates the temperature of the cupboard, and that of the top drawer is given by  $t_1$ . Three temperatures, with differences of about 8° C., are obtained by this apparatus, and this is a desideratum in paraffin imbedding.

Fig. 22 shows the subsidiary parts of the apparatus, and these are intended for the drawers and for the cupboard; their use is too obvious to require description. One point in connection with the shape of the drawers, &c. may be alluded to and that is the sloping back and sides of the trays and drawers. This has some practical value, for when the paraffin has set it allows the whole lot of blocks to be taken out with ease.

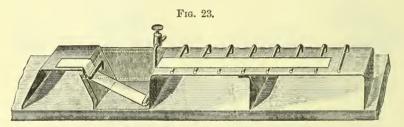
Cutting and After-treatment of Paraffin Ribbon Sections.\*—Dr. G. C. van Walsem advocates the use of hard paraffin to which about 5 per cent. of cera flava has been added for cutting sections with the Minot-Zimmermann microtome. To this instrument, which is preferred

\* Zeitschr. f. wiss. Mikr., xi. (1894) pp. 207-36 (4 figs.).

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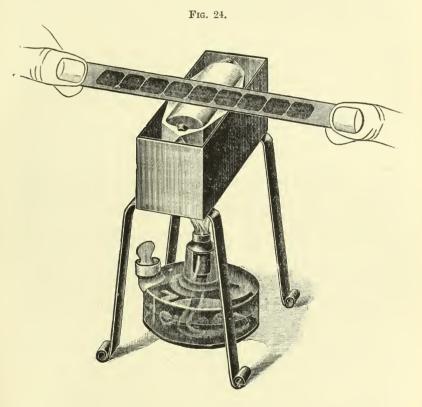
to the Degroot or Reinhold-Giltay microtomes, the author has adapted some subsidiary apparatus for facilitating the cutting of ribbon sections. The first of these is an arrangement for warming the knife. This consists of a kettle, heated by a spirit-lamp, which is placed on the righthand side of the microtome. To the spout is connected a long rubber tube, passing along close to the back of the knife, its other end dropping into a vessel on the left of the microtome. The steam passing through the tube is found to impart sufficient warmth to the knife to prevent the sections curling, and also to render cutting more easy. The knife is placed at such an angle that the sections do not touch the blade, but are passed on to the ribbon frame. By a simple device the microtome is worked with a treadle, thus leaving both hands free for manipulation.

The sections, which have been received on long strips of thin parchment paper, are then immersed in 70 per cent. spirit, preparatory to being stretched. During this process the sections not unfrequently get lifted by the development of air-bubbles underneath, and to get rid of these the author uses a special apparatus (fig. 23). As will be seen it is a pan having a plate on either side, that on the right 36 cm. long, being divided up by wire partitions 0.5 cm. thick and 4 cm.



apart. The pan, filled with 70 per cent. spirit, is] 6 cm. high and its bottom 10 cm.  $\times$  10 cm. The right side is vertical, the left slopes. The plate on the left has a leg inside the trough parallel to the oblique side, and can therefore be moved to and fro. To this leg is soldered a wire at such a distance that it is about 0.5 cm. below the level of the fluid (see fig. 23). On the right side is another wire parallel to and 0.5 cm. above the bottom of the trough. This is also movable to and fro by means of a screw. By passing the strips of paper, which have been placed on the long plate, underneath the two wires and through the fluid, a perfectly smooth surface is obtained. If necessary they can, when they are lying flat on the left plate, be cut up into strips. The strips are then passed rapidly over a roller placed in a vessel filled with water heated to 60°-70° C. (fig. 24). The apparatus is merely a copper tank in which a glass or porcelain roller is placed. The measurements of the tank are  $12 \times 5 \times 5$  cm., and the roller projects 0.5 cm. above the top. By passing the bands to and fro as indicated in the illustration, the sections are damp-stretched.

As a solvent to the paraffin the author expresses his preference for benzin, and then goes on to consider how the section should be stuck. Two great divisions are made : (1) where the sections are to be examined unstained or where the object has been stained *in toto*. (2) Where the sections are to be stained. Under (1) an adhesive of the following composition is given: turpentine oil 1, 20 per cent. aqueous solution of gelatin 2. This makes an emulsion which may be spread on with the tingers. When the sections are placed on this layer they are to be pressed down with filter-paper and then allowed to dry in the air. This procedure is only intended for sections which are not large or of such a nature that they do not crumple, &c. when treated with the paraffin solvent. In such case the section-strips must undergo a previous treatment, and this at the stage when they are removed from the ribbon frame just after



cutting. The one surface must then be covered with a layer of 20 per cent. gelatin solution. When dry the strips are placed in 50 per cent. spirit and then damp-stretched. By this they are made to adhere to the strips, after which, having been dried in air, they are placed in benzin. When the sections are to be stained the adhesive recommended is turpentine oil 1, 2 per cent. aqueous solution of bichromate of potassium 1, 20 per cent. aqueous solution of gelatin 3. This emulsion must always he freshly prepared. When a soft and flexible underlay is requisite, then the slides must be coated with a 10 per cent. solution of guttapercha in carbon disulphide, and when the disulphide has volatilized a 124

coating of Ol. Ricini 3 vols., absolute alcohol 2 vols., is laid on. In this way very large sections can be laid on evenly after smoothing them down by pressing on several folds of filter-paper. If now placed in absolute alcohol the strips come away, leaving the sections adhering.

Many more details are given by the author as to the treatment of paraffin sections, but we have only space to indicate the more salient features of his procedure.

#### (4) Staining and Injecting.

Staining Nervous System of Insects.\*—M. A. Binet has obtained his best results by using sublimate and osmic acid as fixing agents; certain parts of the dotted substance take on a deep stain, under the influence of osmic acid, and this stain distinguishes them from other regions, and allows of the delimitation of certain important lobules. The best fluid in which to make a rapid dissection of the nervous system is the blood of the insect itself. Permanganate of potash will reduce sections which have been blackened too deeply by osmic acid, and care must be taken, as too long treatment will result in the complete transparency of the sections.

Like others, M. Binet has found great use in corrosive sublimate. Pieces fixed with it may, after the method communicated to the author by M. de Nabius, be treated for a day with a 1 per cent. solution of sulphate of copper; they should then be washed for six hours, and then stained for twelve in a solution of 0.05 gr. hæmatoxylin, 15 ccm. of absolute alcohol, and 25 ccm. of distilled water; there should then be another bath of the same solution of sulphate of copper, and dehydration by various strengths of alcohol.

Staining Nervous System of Embryonic Crustacea.<sup>†</sup>-Mr. E. J. Allen found that embryos of the Lobster were the most convenient for his purpose, as the fibres are coarser than in most embryonic Crustacea, and the thoracic ganglia can be exposed with comparative ease. He is in the habit of keeping a standard solution of methylen-blue of 1/10 per cent. in normal salt solution; this is diluted with 15 or 20 volumes of a mixture of three parts of sea-water to one of fresh immediately before The staining is most satisfactory at a temperature of 20° to 25° C. use. The embryos are placed on a slide with the thoracic ganglia uppermost, The process of staining and covered with methylen-blue solution. may be watched under the Microscope; no cover-glass should be used. The preparations may be fixed in a solution of ammonium picrate containing an excess of ammonium carbonate, and mounted in glycerin diluted with an equal volume of the fixing solution. They do not appear to be quite permanent, and the best way is to examine a very large number of fresh preparations. In dealing with methylen-blue it must always be remembered that one cannot be sure that the whole of the element has taken up the blue.

Staining of Myelin and Fat by Osmic Acid or Tannin.<sup>‡</sup>—Dr. L. Azoulay has tried to fix osmic acid on the myelin of nervous tissue or on fat, and then to reduce it by tannin or its analogues. To do this he

‡ Anat. Anzeig., x. (1894) pp. 25-8.

<sup>\*</sup> Journ. de l'Anat. et de la Physiol., xxx. (1894) pp. 468-74.

f Quart. Journ. Micr. Sci., xxxvi. (1894) pp. 461-4; 483-5.

makes very fine sections of pieces which have been for some months in Müller's fluid; after putting them in 90 per cent. alcohol he washes them lightly with water to get rid of the alcohol. They are then immersed for from 5 to 15 minutes in a 1/500 or 1/1000 solution of osmic acid, washed in water, and then immersed in a solution of 5 per cent. or 10 per cent. tannin, and heated to 50° to 55°; after about 5 minutes they may be washed several times in water, have a double stain or carmine or watery solution, and be mounted in the usual way.

The sections thus treated are brown or black in the white substance, and grey in the grey; it is only the myelin that is stained. An advantage of this method is that, the coloration not being diffused as by the Weigert or Weigert-Pal method, the fine structure of the fibres can be studied.

The sections should be thin; if they are too thick they should, after the final washing in water, be decolorized by Pal's method.

Another method is to take fine sections from the alcohol in which they have been lying and to wash them lightly with water, put them in a warm bath of 5 or 10 per cent. tannin for 3 to 10 minutes, wash in water, decolorize or not, wash for a long time in water, double stain or not, and mount in the ordinary way. This method is simple, rapid. safe, and cheap. Could more be demanded of it?

Staining of Centrosomes.\*-Herr G. Karsten states that the same staining reagents are not applicable for bringing out the directing spheres (centrosomes) in the case of plants as of animals. No one reagent has been found to answer in all cases. The best results were obtained by the following method of treating vegetable cells :- The material was fixed either by a mixture of 0.5 gr. chromic acid and 0.2 gr. osmic acid in 100 of water, or by one of 0.5 gr. platinum bichloride and 0.5 gr. chromic acid in 100 of water, or by absolute alcohol. The staining reagent used was a mixture of Weigert's acid fuchsin and Grubler's methyl-green 0.0. After allowing a sufficient period, the centrospheres and protoplasm are coloured red or rose, while the nuclear chromosomes retain their green colour even after washing with absolute alcohol. Coccinin may also be used with advantage for staining the protoplasm and the centrospheres.

Staining Reaction of Sputum.<sup>†</sup>—Dr. C. Zenoni, not being satisfied with Schmidt's method for the differential diagnosis of pneumonia and bronchitis, adopted a saturated solution of safranin instead of the Biondi triple stain, which imparted to mucoid bronchitic sputum a greenish-blue hue, and to the pneumonic a red, tending to violet. With the safrauin the mucoid elements are stained sulphur yellow to brownish yellow, the albuminous red or reddish yellow. The cover-glass preparations are first fixed for 1/4 hour in alcohol, and then stained with a semi-saturated solution of safranin. Leyden's spirals showed a difference of colour in the central and outer parts of the spiral. According to the author, the method is very good for making a differential diagnosis between bronchitic and pneumonic sputum, even on macroscopic examination.

\* Journ. de Bot. (Morot), viii. (1894) p. 245. † Centralbl. f. wiss. Med., xv. (1894) p. 257. See Centralbl. f. Bakteriol. u. Parasitenk., xvi. (1894) p. 667.

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'Eosinophilous Cells'of Gonorrhœal Pus.\*-Dr. G. Caneva stains coverglass preparations of gonorrhœal discharge with a saturated solution of eosin, and in this way is able to distinguish two kinds of eosinophilous elements, the difference consisting in the size of the granules. The first kind of eosinophilous cell is of medium size, usually mononuclear, and the granules are about the size of gonococci. These are of rare occurrence in the acute form of gonorrhea. The second sort of eosinophilous cell contains very fine granules, and the cell may be mono- or polynuclear. In the acute form these cells predominate. If the eosin stain is followed by saturated methylen-blue solution the microscopical picture is altered. The rose-red gonococci now become blue and are easily distinguished from the first described granules. If the methylenblue solution be allowed to act longer than five minutes, the second kind of granules become decolorized and the decoloration is hastened by the addition of phenol or alcohol to the staining solution. The gonococci are invariably found in the eosinophilous cells of the second kind and never in the first. In cases of chronic gonorrhœa the number of cells of the first kind and of free gonococci increases. The latter are stainable by Gram's method if, instead of using alcohol as a decolorizer, anilin oil be mixed with an equal volume of xylol or oil of cloves.

Media for distinguishing between Bacillus typhi abdominalis and Bacillus coli communis.<sup>†</sup>—Herr Marpmann has found that media to which reduced pigments have been added are extremely suitable for diagnosis, and has applied this method for distinguishing between Bacillus typhi abdominalis and Bacillus coli communis. In the first series 1·grm. of fuchsin is dissolved in 100 parts of water and decolorized with sodium bisulphite solution, and after having been mixed with 2 per cent. agar or gelatin is placed in test-tubes and sterilized. The red colour reappears on the addition of an aldehyde, and as the same reaction occurs after inoculation with certain cultures, it would seem to follow that these microbes produce aldehyde.

In the second series malachite-green is used instead of fuchsin, and this pigment appears to be more suitable for the purpose, as with fuchsin it is necessary to add a greater quantity of bisulphite, which is sometimes detrimental to cultures. Malachite-green, which in itself is very sensitive, is better used in conjunction with agar than with gelatin, and affords a means of discriminating between different species of bacteria by the colour of the growth, that of *B. typhosus* being dark green, and that from *B. coli com.* being greyish white. Vibrio choleræ, V. Metchnikovi, *Bac. liquefaciens*, *B. typh. murium*, are green, while Spirillum rubrum and some cocci and Saccharomycetes are colourless.

In a third series agar was blackened by means of indulin or nigrosin, aqueous solutions of these pigments being added until the agar became non-transparent. On such media micro-organisms grew well, and differences were observable between the typhoid-like bacteria.

Staining Anthrax.<sup>±</sup>—Dr. Johne gives a method for staining the gelatinous sheath of anthrax bacilli, and the procedure is as follows:—

\* La Riforma Med., 1894, No. 25. See Centralbl. f. Bakteriol. u. Parasitenk., xvi. (1894) p. 654. † Centralbl. f. Bakteriol. u. Parasitenk., xvi. (1894) pp. 817-20.

<sup>‡</sup> Deutsche Tierärztliche Wochensch., 1894, No. 35. See Centralbl. f. Bakteriol. u. Parasitenk., xvi. (1894) p. 871. The cover-glass preparation, dried in air, is covered with 2 per cent. aqueous gentian-violet solution, and warmed until it begins to vaporize; after this it is washed in water, and next treated with 2 per cent. acetic acid for 6-10 seconds, and then washed in water again.

New Staining Process.\*—Dr. O. Zacharias recommends the following process for both animal and vegetable preparations. The material is laid in 70 per cent. alcohol, and then, for from 16 to 54 hours, in aceticearmine, prepared by boiling 1 gr. powdered carmine for 20 minutes in 150–200 gr. dilute acetic acid, and filtering when it has become cold. The preparation is washed in dilute acetic acid, and then placed for from 2–3 hours in ferric-oxide-ammonium-citrate.

Staining and Fixing of Diatoms.<sup>†</sup>—Dr. P. Miquel finds the staining reagent best adapted for demonstrating the gelatinous envelope of diatoms to be an aqueous or boric solution of methylen-blue, which is not taken up so readily by the gelatinous stipe. The same reagent, especially in a slightly ammoniacal solution, may be used for demonstrating the nucleus, which is stained blue, while other substances contained in the cell take from it a dark blue-violet stain. For fixing, the author uses a solution of 65 gr. corrosive sublimate and 15 gr. sodium chloride in 100 ccm. of water.

Easy and Rapid Method for Removing Picric Acid from Tissues. -Herr O. Jelinck uses both pieric acid alone and in conjunction with sublimate as a fixative. In the first case it is a saturated aqueous solution, in the latter it is a saturated aqueous solution of pieric acid with an equal bulk of a saturated solution of sublimate in physiological salt The piece of tissue should be very small, and immersed in solution. 30-50 times its bulk of fluid for 1-24 hours. To 100 ccm. of the fluid it is often useful to add 5 ccm. of acetic or formic acid. When properly fixed the pieces are to be transferred to alcohol, the strength of which must be gradually increased until absolute alcohol is reached. The pieces should be often moved about and the alcohol frequently renewed. By transferring the sections (celloidin sections) to a saturated solution of carbonate of lithia the picric acid is easily dissolved out. Afterwards they are washed in distilled water and stained in the usual way with hæmatoxylin or other dyes.

By a similar process pieric acid may be removed from comparatively large pieces. In this case it is best to proceed as follows :--To a saturated aqueous solution of lithium carbonate add a few drops of 95 per cent. alcohol until a faint white precipitate falls. In this turbid fluid immerse the object to be fixed. Then keep on adding to the fluid, which has now become clear, solution of lithium carbonate until the precipitate no longer dissolves, and alcohol, which of course must be frequently changed so long as the yellow colour shows itself. The tissue or piece then appears white, just as if it had been fixed in sublimate. Last of all transfer it to 95 per cent. spirit to free it from the last traces of lithia, and thereupon treat in the usual way.

\* Forschungsber. Biol. Stat. Plön (Zacharias) Pts. 1, 2, 1893, 94. See Bot. Centralbl., lx. (1894) p. 137. † Le Diatomiste, ii. (1894) pp. 107, 112.

‡ Zeitschr. f. wiss. Mikr., xi. (1894) pp. 342-6.

Neutral Red.\* - According to Prof. Ehrlich this new pigment. neutral red, is excellently adapted for biological researches and vital staining, as it possesses a striking affinity for living tissues. If tadpoles be placed in solutions of 1-10,000 up to 100,000 the animals become stained in quite a short time, and during the first and second day of their immersion absorb so much of the pigment that all their tissues become dark red. The pigment may be seen in the cells as minute granules. Larger animals may be subcutaneously injected, and even feeding with the pigment gives good results. In germinating plants the author obtained successful staining results, and by combination with other pigments, e. g. methylen-blue, &c. a double or triple staining.

Demonstration of the Presence of Iron in Granules of Eosino-phile-Leucocytes.<sup>†</sup> — Dr. L. F. Barker, after noticing Dr. A. B. Macallum's # method for the demonstration of iron in chromatin, gives the following account of his own method for the micro-chemical demonstration of iron. "In my experiments cover-glass preparations, such as are employed for the colour analysis of the leucocytes according to the methods of Ehrlich, were heated on the copper bar at a temperature of 120° C. for from one to two hours, and were then treated in the following way :--- A drop of solution of ammonium sulphide, prepared just before using, was placed upon the smeared surface of the cover-slip, and this was immediately laid upon a drop of glycerin, the glycerin and sulphide solution mixing, upon a large thick glass slide. The preparation was then placed in the thermostat at 60° C. Once as early as after 6 hours, but usually at the end of 24 hours, and more markedly at the end of 48 hours, the greenish-black iron reaction in chromatin of the nuclei of the white corpuscles was apparent in the specimens. By this time the hæmoglobin of the red corpuscles had assumed only a slight greenish tint. In an occasional leucocyte, however, granules of the size and shape of the eosinophile granules were very distinctly stained yellowish-green.

To make sure that the granules were really those of the eosinophile leucocytes (although the morphology of these granules is in itself so typical that they can, as a rule, be recognized in fresh unstained specimens of blood), some cover-slip preparations, known by control-studies of slides stained with the triple stain to contain a much larger number of eosinophile leucocytes than normal, were submitted to the same test. In these too, the eosinophile granules stained sharply. The blood taken from a patient whose blood contained 18 per cent. of eosinophiles yielded very striking pictures.

The granules in the sulphide-glycerin preparations do not assume quite the same tint as do the nuclei of the leucocytes; the latter are stained greenish-black and have a dull appearance. The eosinophile granules, by contrast, are more highly refractive, and while stained greenish-black show also a slight yellowish tint.

Osmic-Iron-Hæmatoxylin Staining Method.§-Dr. Kaiser treats the central nervous system in the following way :- The pieces are placed

\* Allgem. Med. Centralzeit., 1894, p. 20. See Zeitschr. f. wiss. Mikr., xi. (1894) 250. † Bull. Johns Hopkins Hospital, v. (1894) p. 93. † Proc. Roy. Soc., l. (1892) pp. 277–86. See also this Journal, 1891, pp. 82–9. p. 250.

§ Neurol. Centralbl., xii. (1893) pp. 363-4. See Zeitschr. f. wiss. Mikr., xi. (1894) pp. 249-50.

in Müller's fluid, and after two or three days they are cut down to slices of 1-2 mm. thick. These slices are immersed in Marchi's fluid (Müller's 2 parts, 1 per cent. osmic acid 1 part) for 8 days. After removal they are washed, then hardened in alcohol, and imbedded in celloidin. The sections are now placed for about 5 minutes in a mixture of Liq. ferri perchlor. 1 part,  $\hat{H_2O}$  1 part, rectified spirit 3 parts, and then in Weigert's hæmatoxylin solution, which is heated for a few minutes. The fluid must not boil, as the celloidin is easily crumpled. After washing in water they are differentiated by Pal's method. The oxalic acid is neutralized by washing in ammoniated water, by which the colour is often intensified. The nerve-fibres are stained dark brown or black. The nuclei of the ganglion cells are a blackish brown.

Ehrlich's Triple Stain.\*-Dr. G. Reinbach gives a formula for the triple stain as improved by Ehrlich. The author used it for staining cover-glass preparations of blood. Its composition is as follows:---Saturated aqueous solutions of orange G, 120 grm.; acid rubin, 80 grm.; methyl-green, 100 grm.; H<sub>2</sub>O, 300 grm.; absolute alcohol, 180 grm.; The aqueous solutions must be saturated. The glycerin, 50 grm. mixture is not to be shaken, but the necessary quantity should be pipetted off. The fixed cover-glass film is treated with the solution for 5 to 10 minutes; the superfluous stain is washed off with distilled water, the surface dried with blotting-paper, and the preparation mounted in balsam.

Flagella-Staining.+-Herr R. Bunge obtained very good results from using cultures which had been incubated for 24 hours and then kept at the room temperature for another two days; even cholera spirilla were very successful. The preparations must not be too strongly fixed with heat. Instead of carbol-fuchsin, carbol-gentian-violet was used. The mordant used was that already noticed in this Journal.<sup>‡</sup> One very important result of the author's experiments was that a differential diagnosis between B. typhosus and B. coli commune could not be made by the number of flagella, for the latter microbe often had more than the former. The author was also able to demonstrate at the same time in the organisms examined by him, Proteus, B. coli, B. typh., cholera, subtilis, both flagella and capsules. The method was as follows :---The cover-glass prepara-tions are first treated as already described.§ They are then immersed for 1/2-1 minute in 5 per cent. acetic acid, after which they are washed and dried. The preparations are thereupon mordanted three or four times, the washing being done after the manner of Nicolle and Morax. This over, the preparations are dried, and then stained with gentianviolet; this step is followed by 1/2-1 minute in 1 per cent. acetic acid. After this, they are washed in water and dried. A three days' old culture is best suited for this procedure.

BOECK, C .-- Neues Verfahren bei der Färbung der Mikroparasiten auf der Oberfläche des Körpers. (New Process in Staining Micro-parasites on the Surface of the Body.) Mtsh. f. Prakt. Dermatol., 1894, pp. 467-70.

\* Arch. f. Klin. Chirurg., xlvi. (1893) pp. 486-562 (1 pl.). See Zeitschr. f. wiss.

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BUNGE, R.--Ueber Geisselfärbung von Bakterien. (Staining Flagella of Bacteria.) Fortschr. d. Med., 1894, pp. 462-4.

VINCENT, H .- Sur un nouveau mode de coloration des microorganismes dans le sang. (On a new Means of Staining Micro-organisms in the Blood.) Compt. Rend. Soc. Biol., 1894, pp. 530-1.

(5) Mounting, including Slides, Preservative Fluids, &c.

Preservation of Sea-weeds.\*-Dr. J. P. Lotsy recommends the following method of preserving specimens of Florideæ, which prevents swelling of the cell-walls or contraction of the protoplasm, and preserves the chromatophores uninjured. The specimen is first laid in a 1 per cent. solution of chrome-alum in sea-water, and kept there for a period varying from 1 to 24 hours, according to the size and texture of the species. The chrome-alum is then completely washed out, and the specimen placed in a mixture of 5 ccm. of 96 per cent. alcohol in 100 ccm. water, and vigorously stirred. The amount of alcohol is then increased by increments of 5 ccm. every quarter of an hour until it amounts to 50 ccm. The specimen is then removed, and placed in a mixture of 25 per cent. alcohol in distilled water, and the quantity of alcohol again increased in the same way till it amounts to 50 ccm. alcohol to 100 ccm. of water. The same process is again repeated with 50, 60, 70, 80, and 90 per cent. solutions of alcohol in distilled water; the specimens being finally preserved in the last.

New Fixing-material.<sup>†</sup>—Under the name "chrome-potash-sublimateglacial-acetic-acid" (Chromkali-sublimat-Eisessig) Herr Zenker recommends a fixing material for vegetable tissues which has the advantage of penetrating the tissue readily, without producing any shrinking. Its composition is as follows :- 100 parts distilled water, 5 sublimate, 2.5 double potassium chromate, 1 sodium sulphate, 5 glacial acetic acid. It may also be used for preparations of the nervous system.

#### (6) Miscellaneous.

Cobalt-test for Transpiration.t-The property of salts of cobalt of changing in colour according to the degree of moisture in the air, is employed by Prof. E. Stahl to determine the intensity of the transpiration from leaves. A useful material for that purpose is Swedish filterpaper which has been soaked in a 5 per cent. solution of cobalt chloride. This, when dry is intensely blue, passing, when moistened, through all shades to pale red. The freshly gathered leaf, or other part of the plant, is placed between two glass plates covered with freshly dried cobaltpaper. The amount of vapour given off by the leaf is shown by the extent of change in the colour of the paper.

Action of Chemical Reagents on Vegetable Spermatozoids.§-M. W. Belajeff states that if spermatozoids (of Characeæ) are subjected to a 10 per cent. solution of sodium chloride, the central portion is quickly dissolved; if previously stained with iodine-green, a rapid

<sup>\*</sup> Bot. Centralbl., lx. (1894) pp. 15-6.

<sup>&</sup>lt;sup>1</sup> Münch, Med. Wocheuscher, xxvii. (1894) p. 532. See Bot. Centralbl., 1λ.
<sup>94</sup> ) p. 45. <sup>‡</sup> Bot. Ztg., lii. (1894 1<sup>6</sup> Abtheil., pp. 118-9.
<sup>§</sup> Flora, lxxix. (1894) gänzungsbd., pp. 40-3. (1.94) p. 45.

swelling of the coloured portion is observed. The anterior and posterioends are unchanged, as well as the cilia, but the two ends remain connected by a fine thread. If the same solution is applied to the spermator genous cells, the nuclei swell up, and finally are dissolved.

If the spermatozooids are immersed for 24 hours in 0.5 per cent. hydrochloric acid, the central portion contracts, though it is still stained by iodine-green; the number of coils is reduced from  $2\frac{1}{2}$  to scarcely one. The anterior portion and the cilia undergo no essential change; numerous refringent granules or drops appear in the swollen posterior portion. This solution produces but little change in the spermatogenous cells.

In a mixture of 1 part pepsin-glycerin and 3 parts 0.2 per cent. hydrochloric acid, after 24 hours the anterior end and the cilia of spermatozoids are still retained within the mother-cell disappear; the central portion and the coils become narrower and closer. If free spermatozoids are placed for some minutes in gastric juice, the anterior end becomes finely granular, the cilia disappear, the central portion becomes shorter and strongly refringent, the posterior end swells and becomes coarsely granular.

In a fluid containing trypsin prepared after Kühne's method, the spermatozoids are finally completely absorbed, the process beginning with the central portion.

These chemical reactions point to the same conclusion as the staining reactions, viz. the correctness of Belajeff's view,\* that the central portion only of the spermatozoid is derived from the nucleus of the mother-cell, the anterior and posterior ends and the cilia from the cytoplasm.

Determination of Coniine and Curcumine.<sup>†</sup>—Herr A. Rossol gives the microchemical reactions, and the mode of demonstrating the presence of these two substances, the former in the tissues of the hemlock, the latter in the rhizome of *Curcuma longa*. Coniine he finds especially in the meristem of the growing point, and in the parenchyme of the sieveportion of the vascular bundles. The action of potassium biniodide causes a red-brown precipitate soluble in sodium hyposulphite. Curcumine occurs in the parenchyme of the rhizome dissolved in an etherial oil. It is nearly insoluble in cold water and in glycerin, soluble with difficulty in hot water, in benzol, and in carbon bisulphide, readily soluble in alcohol and in ether, the latter solution showing a green fluorescence. Alkalies dissolve it with a red-brown, acids with a carmine-red colour; this reaction with concentrated or moderately dilute sulphuric acid is the most useful.

Sounding for Diatoms.<sup>‡</sup>—In the most recent of his series of papers on the technique of diatoms, M. J. Tempère describes the best modes of sounding (sondages) for diatoms, and of extracting them from the material thus obtained. When mud or sand has been obtained by dredging, the best mode of determining the presence of diatoms is the system of floating (*flottage*). The material is repeatedly agitated in water in a basin, and the scum which forms on the surface ladled off by

† JB. Nieder-öst. Land-Öberrealsch. Wiener-Neustadt, 1894. See Bot. Centralbl., lx. (1894) p. 174. Le Diatomiste, ii. (1894) pp. 122-5.

<sup>\*</sup> Cf. this Journal, 1893, p. 662.

a spoon. In this will be found the diatom-frustules, supported by the air which they contain, and they have then simply to be separated by repeated filtering.

Isolation of Rennet from Bacteria Cultures.\*-Prof. H. W. Conn describes seven bacteria which possess the power of producing a rennet ferment in large quantities. For the detailed description of the microbe the original should be consulted. The method of isolating the ferment is as follows :--- "The bacteria in question are cultivated in milk for several days, and in some cases for two weeks. By this time the curd is precipitated and at least partially dissolved, and the result is a somewhat thick liquid containing of course immense numbers of bacteria. This liquid is filtered through a porcelain filter to remove the organisms, and a clear, usually amber coloured filtrate is thus obtained. The filtrate contains in solution all of the soluble chemical ferments which may have been formed by the bacteria. This filtrate is now acidified with H<sub>2</sub>SO<sub>4</sub> and then common salt added to a state of supersaturation. When this condition is reached there appears on the surface of the liquid a considerable quantity of snow-white scum. This scum is removed from the liquid, purified if necessary by precipitation, and then dried. It produces a snow-white powder, which upon experiment is found to be active in this curdling action upon milk, and to have all the essential characters of rennet. The ferment thus obtained is not chemically pure. containing, besides the rennet ferment, a varying amount of the tryptic ferment formed at the same time; but the rennet ferment is most abundant and very active. This ferment can be kept indefinitely, is killed by heat, acts best at a temperature of 30-35° C., and curdles sterilized milk under proper conditions in half an hour. Experiment shows that no organisms are present in the curdled milk, and there is thus no doubt left that we are dealing with a chemical ferment similar to rennet, and which is produced by the growth of these micro-organisms The ferment does not appear to be exactly identical with in milk. rennet, some of its chemical tests being different. This may be due to the impurities which are present or to an actual difference in the ferment."

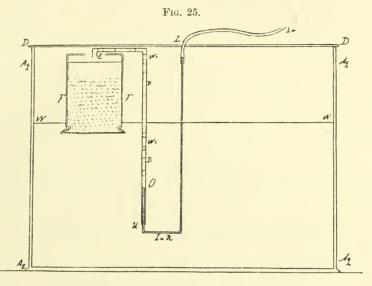
Buttersack's Vaccinia Microbe.<sup>†</sup>—Dr. A. Dräer has examined preparations made from vaccine pustules, serum, blood, saliva, and egg albumen, for certain forms described by Buttersack as occurring on vaccinia pustule. In 64 per cent. of the preparations the Buttersack body was present, and the author comes to the same conclusion as Landmann, viz. that these appearances are entirely artificial, and can be produced in any albuminous fluid by the method of preparation.

The method was to treat cover-glass films for 15 minutes with a 7 per cent. nitrate of soda solution, and then for an equal length of time with 5 per cent. sulphuric acid. The cover after having been washed for an hour was dried, placed on a slide, and examined. The cover was fixed by small bits of wax, and the medium through which the preparation was viewed was, of course, air.

\* Science, xx. (1892) p. 1892; and also Fifth Report of the Storrs School of Agriculture, 1892, pp. 106-26.
† Centralbl. f. Bakteriol. u. Parasitenk., xvi. (1894) pp. 561-4.

Aquarium Filter.\*-Prof. R. v. Lendenfeld has devised a simple method for filtering sea water in an aquarium without pouring out the water and without disturbing the sand layer at the bottom.

In the aquarium  $A_2$  (fig. 25) which is closed with a cover, the level of the water stands at W. For freshening the water air is introduced by the tube L u through the hole L in the cover, and passing down the thin tube L u R rises in bubbles from the end O. The end of the thin tube

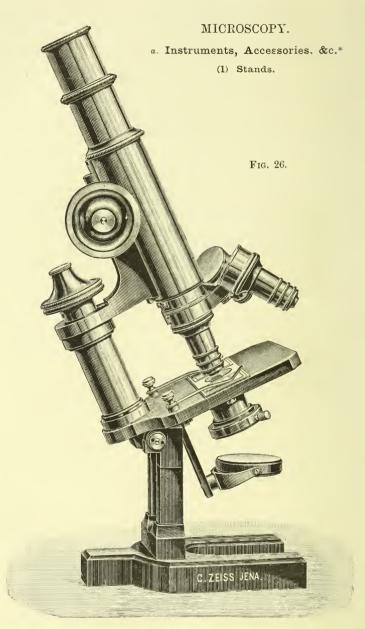


L u R passes up into a wider tube W r, which after a double bend dips into the filter F. In this tube the air-bubbles rise and carry with them water which enters at its lower end and flows out at E into the filter.

By regulating the stream of air it is possible to regulate the amount of water so that the filter shall always be nearly full, but not run over.

Ink-Crystals.<sup>†</sup>-Dr. E. Trouessart says that ink-crystals can be procured by allowing a drop of ink to dry on a slip of glass, and be seen by powers magnifying from 50 to 200 diameters. Different inks give somewhat different crystals, but the chemical composition of none of them is yet exactly known.

- \* Zool. Anzeig., xvii. (1894) pp. 431-2.
  † See Nature, li. (1894) pp. 60 and 1 (1 fig.).



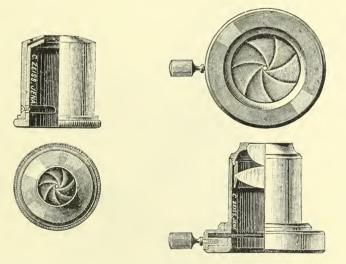
\* This subdivision contains (1) Stands; (2) Eye-picces and Objectives; (3) Illuminating and other Apparatus; (4) Photomicrography; (5) Microscopical Optics and Manipulation; (6) Miscellaneous.

Zeiss Stand VIa.—Messrs. Zeiss have designed a stand which they consider particularly suitable as a small laboratory and travelling Microscope.

The stand (fig. 26) is on the lines of their usual Microscopes. With respect to size and equipment it stands just midway between stands IV. and VI. The upper body of the instrument is inclinable as a whole and may be tilted into a horizontal position. The solid metal stage has a surface of  $80 \times 80$  mm.  $(3\frac{1}{8} \times 3\frac{1}{8}$  in.). It is therefore large enough for the convenient manipulation of the usual forms of object-slides. Below the stage, in the axis of the Microscope, is fixed a sleeve into which any of the following interchangeable appliances may

Fig. 27.

FIG. 28.



be inserted, viz.—the ordinary cylinder diaphragm (with three stops of different apertures); or the new form of iris cylinder diaphragm (fig. 27); or the illuminator No. 19 (fig. 28), of numerical aperture  $1 \cdot 0$ , with iris diaphragm centrally fixed in the lower focal plane of the condenser. The illuminator suffices for bacteriological work with so-called "full illumination."

For central illumination, the aperture of  $1 \cdot 0$  will be found sufficient for all purposes, but the condenser is not adapted for observation with oblique illumination.

The light is reflected to the condenser by means of a plane and concave mirror of 36 mm.  $(1\frac{3}{8}$  in. diam.), attached to an arm which is movable in all directions.

The prism which forms the guiding element for the micrometermovement or fine-adjustment, rises from the top of the stage. This micrometer-movement is, Messrs. Zeiss state, well adapted for work with the highest powers.

The tube is not graduated, and when extended to its full length, has 1895 Q

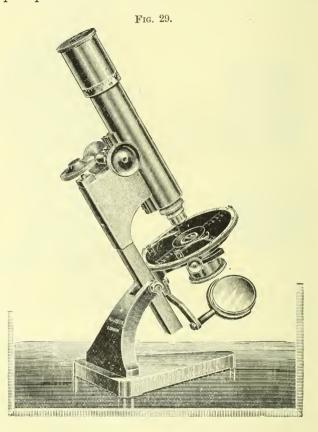
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the exact length of 160 mm. for which all their objectives have been adjusted. When a revolver is used, which is a useful adjunct to this stand, the tube should be shortened 15 mm., which is the depth of the collar of the revolver. The correct position of the tube when a revolver is used may be seen by a mark cut into the draw-tube.

Messrs. R. & J. Beck's New Student's Petrological Microscope.— Figs. 29 and 30 illustrate two forms of a cheap Petrological Microscope recently made by Messrs. Beck.

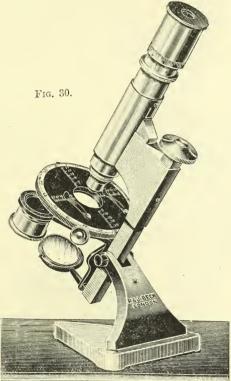
The only difference in the construction of these two instruments is that one has a rack-and-pinion, and the other a sliding coarse-adjustment.

The principal feature in the construction of a small model Microscope



is the fine-adjustment, which in these instruments, the makers affirm, is exceedingly simple, yet perfectly efficient.

In the present instrument the stage is supplied with a centering adjustment, revolves concentrically with the optic axis, and has a graduated scale of degrees on the circumference, with finders in horizontal and vertical directions. The polarizer is fitted to an arm in the substage which swings in and out, and has a revolving fitting with spring catches to hold it at the points when the prisms are crossed; an achromatic system of lenses is supplied to fit in over the polarizer, and is easily adjusted in the sleeve for axial illumination.



The analyser is fitted over the eye-piece, which is supplied with cross wires and is in a rotating fitting similar to that of the polarizer; being provided with spring detents, it revolves freely over the eye-piece, and has a graduated scale of degrees on its circumference.

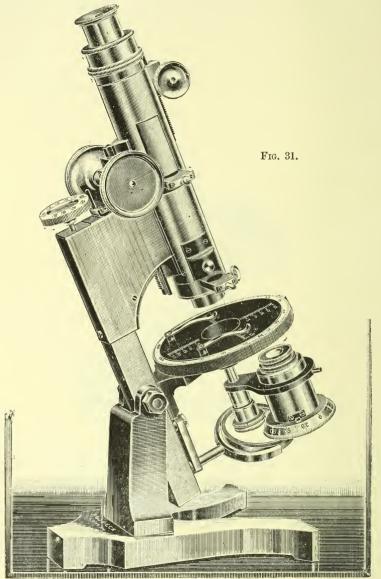
Messrs. R. & J. Beck's Large Model Petrological Microscope.— This instrument has been specially designed for advanced petrological work (fig. 31).

It has a rack-and-pinion coarse-adjustment and micrometer screw fineadjustment. The draw-tube carries a slide containing a Bertrand lens, which can be pushed in and out, and is provided with a rack-and-pinion adjustment for focusing it, when examining the rings and brushes of crystals.

The centering arrangement is supplied to the nose-piece, or may be added to the stage instead if desired.

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The revolving stage has a graduated scale of degrees on its circumference and has finders on its surface in horizontal and vertical directions.



The substage is arranged to swing in and out from the central position for polarized or transmitted light, and carries a condenser over

the polarizer for axial illumination; the substage focusing adjustment is by a spiral screw actuated by the small milled head, which in the figure is shown immediately over the mirror.

The polarizing prism is in a revolving fitting which is divided on its circumference; it has a catch to hold prism at points of extinction.

The analyser is mounted in a sliding box, and is fitted in the tube of the instrument; beneath the analyser is a Klein's quartz plate, also mounted in a sliding fitting, and either can be immediately removed from the axis of the instrument.

The double mirror is arranged on an arm to swing in and out.

New Microscope for Observations at High Temperatures.\* — Mr. Von Wyronboff has had constructed by Nachet a polarization Microscope for the examination of objects at temperatures up to 600°. It is claimed to be simpler and more serviceable for the object in view than the Lehmann instrument, chiefly owing to the long focus given to the objective. The object lies on a perforated ring-shaped sheet of copper which is heated by two Bunsen burners and is attached to a special insulating support to prevent any heat being conducted to the body of the Microscope. As the object therefore cannot be moved, the whole Microscope is made to revolve in a horizontal semicircle and can be also moved horizontally by two guides.

From the description of this wonderful instrument we learn that the "motions provide for the measurement of inclinations due to extinction," and that "the instrument does not exactly measure temperatures, but some thermometric substance."

## (3) Illuminating and other Apparatus.

Substage Apochromatic Condenser with Collar Correction.—At the Society's meeting on March 20th last, Mr. E. M. Nelson read the following note :—"The apparatus before you this evening can hardly be called novel, for it was suggested by me some ten years ago; I believe, however, that this is the first actually constructed. The reason why my first suggestion was not carried out was owing to its being stated on all sides that the game was not worth the candle; but practical results now obtained disprove this statement.

The mechanical arrangement is very simple: the correction collar is similar to that of an ordinary objective, it has a steeper spiral slot, and only half a revolution of movement; a long arm is fixed to the collar so that it may conveniently be reached by the finger (fig. 32). Mr. Powell has constructed it so that it will turn easily and smoothly at the slightest touch. This is an important point; for if the movement were as stiff as the collars on some objectives it would be impossible to move the collar without throwing the condenser out of centre. It is needless to state that the collar moves only the back lens of the combination, leaving the mount rigid.

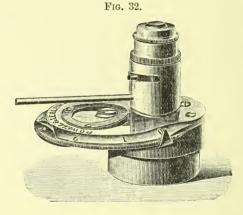
The object of the correctional movement is primarily to increase the maximum aplanatic aperture of the condenser; this is effected by separating the lenses. If the back of a wide-angled objective be examined, when an object is illuminated by the full aperture of the

\* Amer. Micr. Journ., xv. (1894) pp. 352-3.

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condenser, the edge of the flame being in focus, it will be noticed that the illuminated portion of the back lens will be oval and pointed instead of circular. Also that when the condenser is racked up, although the exterior shape of the illuminated portion will become more circular, two dark patches will appear on either side of the centre, showing the operation of the spherical aberration of the condenser. If under these circumstances the lenses are separated by means of the collar adjustment, the black spots will be closed up, and a circular and evenly illuminated disc of illumination of a larger size will be secured.

Here we have a distinct gain, in consequence of which we ought to be able to see more than we have hitherto done. There is an object in my cabinet that has engaged my attention for nearly a quarter of a century, viz. the well-known diatom *Navicula major*. If its "principal view" be examined two vertical stripes will be seen running down the centre of the hoop (fig. 33a). In the early days of my microscopical

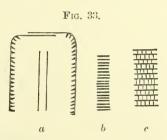


work I happened to be looking at this diatom on a dark ground, using a Gillett's condenser with a circular stop, when I was struck by the blue sheen given out by these two stripes; knowing that this predicated unresolved structure, I attacked it with a new water-immersion 1/8, a lens which Messrs. Powell and Lealand had recently brought out. The illumination was obtained by the same Gillett's condenser with a moonshaped stop, and the stripes were resolved into striæ without difficulty; subsequently these striæ were counted, and found to be 60,000 per inch (fig. 33b), and also they were resolved by an ordinary 1/4 in. of N.A.  $\cdot 74$ .

Now, as it is highly probable that there are no such things as striæ on any of the Diatomaceæ, the structure under discussion probably consists of rows of very minute perforations, and as it has been my endeavour to resolve these perforations, every new improvement in microscopical technique has been tried on this diatom, but hitherto without success. At last, however, the condenser with the collar correction has achieved the feat by means of its enlarged aplanatic solid cone (fig. 33c). This is a very good diatom to work on, because there

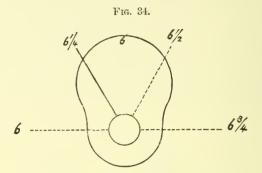
are no edges and raphæ by which false ghosts can be made; it is specially a direct solid cone test, and an oblique azimuthal beam is not of much assistance. This diatom is numbered in Moller's 400 "Typen-Platte" 3.3.11; the hoops are also common in "Sozodont," and the striation will be found pretty constant at 60,000.

In the second place, this correctional collar has another office. It is admitted that an iris diaphragm is exceedingly useful for the purpose of regulating the size of the illuminating cone, but there is this objection to its employment, viz. that it is quite impossible to record the size of the hole or to reproduce a similar sized hole on a subsequent occasion with sufficient accuracy for microscopical purposes. A wheel of diaphragms, or a series of graduated diaphragm discs to drop into a holder, are on this account recommended for critical work, because the diaphragm can always be recorded, and the identical illuminating cone precisely reproduced at any subsequent time.



The disadvantage with this plan is the loss of the power of delicate graduation between any two contiguous sizes of diaphragms, a want which the iris supplies. Now the correctional collar meets this deficiency by making the wheel of diaphragms as adjustable as the iris, in addition to securing an advantage which the iris does not possess, viz. that the size of the illuminating cone can be recorded, and with facility exactly reproduced at any time. For we know that by separating lenses the power of a combination is reduced, and conversely, by closing lenses it is increased, and also that with powers of equal apertures the size of the back lens must be larger with the lower power. Now, if we by means of the correctional collar separate the lenses, we shall reduce the power of the combination, and if the back lens, i. e. the diaphragm aperture, remains the same, the aperture of the combination will be lowered.

Hence we have a very simple means of graduating the apertures between any two contiguous diaphragms; if, for example, we place the lever to the left, so that the lenses may be separated as far as possible, and use a No. 6 diaphragm, and if on examining the object it is thought that the illuminating cone is not large enough, and if when No. 7 is turned on it is found too much, we can go back to No. 6, and by turning the lever 60° towards the right, closing the lenses and increasing the power a little, we shall obtain an aperture somewhere between the No. 6 and 7 diaphragms (fig. 34). This position of the lever may be called  $6_1^+$ ; if this is an insufficient cone we can turn the lever through another 60°, which will further close the lenses and increase both the power and the aperture. The position of the lever may now be called  $6\frac{1}{2}$ ; if that is still not enough we may turn the lever a third 60°, which will bring it home to the right hand, its full movement being a half circle; the position may be called  $6\frac{3}{4}$ . The next step is to turn the lever back to the left, and turn on the No. 7 diaphragm, and so on until the full aperture of the condenser is employed, when the lever must be placed to the left



hand to correct the aberration for the extreme aperture. Thus we can by means of the correction collar graduate the aperture with the same facility as with an iris, and we can record any particular aperture with a degree of accuracy foreign to the iris.

It should be mentioned that it is, of course, necessary to refocus the condenser after moving the collar, just as with an objective."

New Magnifying Lens with Combined Illuminator.—At the Society's meeting on March 20th last, Mr. E. M. Nelson read the following note :—"This magnifying lens or loup is the outcome of a paper I read before this Society on lens mirrors the year before last.\* It consists of a lens mirror with the silver removed in the centre, and another lens placed above it (fig. 35). The focus of the lens mirror is the same as that of the combined lenses, so that the light reflected by the lens mirror is focused on the object when the object itself is in focus.



This is the first Messrs. Watson have made from my design; it is not achromatic, but the curves have been calculated for minimum aberration for the system. The aberration is half that of an equi-convex lens of the same power, viz. 8. The curve of the lens-mirror being fixed, it is not possible with this simple form to reduce the aberration lower. The result is very good and the definition is clear and bright. It is in fact the combination of a loup and a lieberkuhn. The lens mirror not only gives more light, but is cheaper than a speculum, it also forms one of the lenses of the combination.

\* This Journal, 1894, p. 254.

Now as most unmounted objects are seen by reflected light, for it is only prepared objects that are suitable for examination by transmitted light, a lens of this kind is of great service in the field. An object examined by this lens when held up towards a bright part of the sky is strongly illuminated and looks altogether a different thing. For dissecting purposes it is a convenient combined lens and illuminator, and is much easier to manage than other methods now in vogue, such for example as that of Mr. Cheshire which was exhibited here some years ago.\*

The lace-work on the egg of a house-fly can be seen. The object under it this evening is the egg of the parasite of the Ground Hornbill."

Sources of Light for the Projection Lamp.<sup>†</sup>—Herr J. Lützen describes some of the latest projection instruments made in Germany, and gives an account of the various methods of illumination which have been made use of in such instruments. In fig. 36 is shown the  $10\frac{1}{2}$  cm. scioptikon for school work made by Meckel in Berlin. This apparatus is made of the best steel and contains no soldering.

The different methods of illumination can be divided into three groups. Electricity in the form of the arc-light is the most perfect, but on many grounds can seldom be used. Then we have the illumination with oxygen, and lastly without the use of oxygen. Of these two kinds the burners in which a stream of oxygen is used give a light which is nearest to the electric. Illumination without a stream of oxygen, as

FIG. 36.

petroleum light, Auer's incandescent light, or magnesium light, is not to be recommended. The petroleum light is not bright and has a yellow colour; the gas incandescent light does not give sharply defined images, and is not very intense; while the magnesium light burns unsteadily and forms a deposit on the lenses. If these disadvantages, however, could be obviated, this last light would be preferable to any other.

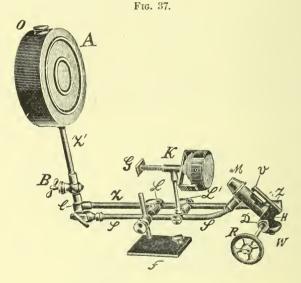
The introduction of the oxygen-burner has had a revolutionary influence in methods of projection. A peculiarity of these burners is that they produce a source of light approximating to the point form, and give a clear quiet white light. When properly constructed they are

\* Carpenter on the Microscope, 9th edition, p. 344, fig. 289.

† Central.-Ztg. f. Optik u. Mechanik, xvi. (1895) pp. 14-6.

without danger and easy to use. The earlier form of burner in which the gases were allowed to mix before burning gave rise to many explosions. In the safety burners, the gases only come together at the moment of burning. They give a little less light than the first, but the loss is more than compensated for by the absolute freedom from danger.

All oxygen burners make use of the Drummond principle, which consists in raising the temperature of burning gas by the introduction of a stream of oxygen, so that a piece of incombustible material placed in the flame is raised to incandescence. The brightness of the light so produced is dependent on the heat of the flame and the emissive power of the incandescent body. As the latter, lime, zirconia, and metallic iridium have been used. Iridium possesses many properties which



should recommend it for such a purpose. It does not oxidise at the highest temperatures, fuses with much more difficulty than platinum, and has a good emissive power. Zirconia is less to be commended owing to its high price and to the fact that it becomes useless after a few hours' burning. Lime as dense as possible and free from sand is in most general use.

As gas for the source of heat, coal gas, hydrogen or the vapour of some volatile inflammable liquid can be employed. Coal gas is usually most convenient. For places without coal gas, petroleum is the usual source of light for projection purposes, but is quite inadequate for demonstrations before large audiences.

The spirit-blowpipe burner, in which the light was obtained by the

introduction of a stream of oxygen into a spirit flame, is not to be recommended owing to its dangerous nature. The most perfect burning material is of course hydrogen, which can be easily transported in a compressed state in steel cylinders. In the Meckel oxy-hydrogen safety burner the light is produced by a plate of lime which emits 500 candles. The use of hydrogen is somewhat expensive and, unless every precaution is taken that it is quite pure, may be attended with danger. As a substitute for hydrogen where coal gas is not to be obtained, the benzine blowpipe burner shown in fig. 37 may be used. The vessel A contains pure benzine which is conducted along the tube L to the heating The latter is heated by a small amount of spirit burning chamber H. in the dish W, so that the benzine is volatilised. The vapour is ignited at the mouth of the blowpipe M. A small amount of vapour passes back through the tube V, and keeps the chamber H hot. Oxygen is introduced by the tube R, and the flame is made to impinge upon the lime K. The small wheel R and the stop-cock B serve to regulate the flow of benzine. This burner gives a light of 200 candles, and is strongly recommended by the author as quite free from danger and convenient in use. Pictures 3 m. in diam. can be obtained with it, which are clearly visible to an audience of 800 people.

Wolff's Hygienic Lamp-shade.\*—Herr J. Rodenstock recommends the Wolff lamp-shade to all who are compelled to work by artificial light. There does not appear to be any very notable peculiarity in its construction. As seen in the figure it serves to protect the head of the observer from the light and heat of the lamp, while it reflects a strong light upon the work-table. At the same time, since the shade only covers part of the lamp, the room is not darkened.

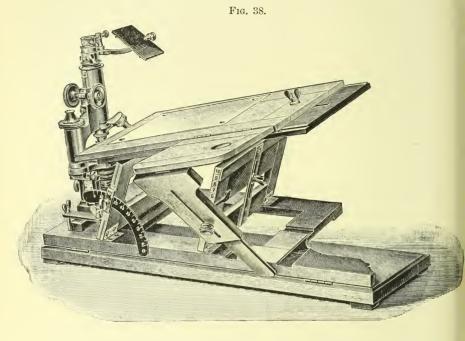
Marking Apparatus for Indicating the Position of Objects or Parts of Objects in Microscopical Preparations.<sup>†</sup>—Prof. S. H. Gage makes use as object-finder of a modified form of the apparatus devised by Dr. May of Philadelphia, and later by Winckel of Göttingen. By means of this apparatus a circular scratch could be made with a diamond point on the cover-glass. The disadvantage of this method is that the line is so fine that is difficult to find, and in the case of homogeneousimmersion objectives is obliterated by the liquid. The author has therefore modified the apparatus by replacing the diamond point by a small brush with which a circular mark can be made on the cover-glass. Its mode of attachment to the Microscope must be given in the author's own words:—" This brush was attached to a piece that could be made eccentric, then this to another, rotating on a central axis, which was screwed into a piece with Society screw, which in turn could be attached to the nose-piece."

Sawing Rock-sections.<sup>‡</sup>—For this purpose the Geological Survey in Washington uses a band-saw, which consists of an endless steel wire 1/8 inch thick, running at a high rate of speed over two fly-wheels.

<sup>\*</sup> Central-Ztg. f. Optik u. Mechanik, xvi. (1895) pp. 25-6.

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Bernhard's Drawing Desk.—A description of this apparatus (fig. 38) appeared in the last number of this Journal, p. 106.



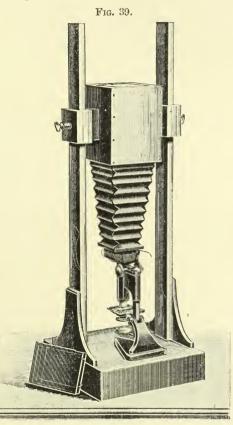
"Loup."—Mr. E. M. Nelson writes, "The word 'loup' has of late years been so much used to express in English a simple Microscope, a magnifying lens of higher power than a reading glass, &c., &c., that I think a note should be inserted about it among new scientific terms when the next list is issued.

It is of course manufactured from the French *loupe* or German *Loupen*. There is no single word in English that exactly expresses the meaning. A loup is a simple Microscope, either achromatic or chromatic, varying in power from 5 to 25 diameters."

# (4) Photomicrography.

Messrs. R. and J. Beck's Vertical Photomicrographic Camera.— This simple form of photomicrographic camera is intended for use with the Microscope in a vertical position, and for several kinds of work it is exceedingly convenient.

The Microscope is easily adjusted and does not require any special attachment to the fine-adjustment. The camera is raised or lowered by releasing the screws which attach the body of the camera to the two uprights. The apparatus is exceedingly rigid, the base being heavily weighted. Each is furnished with one double platc-holder and a fine ground-glass focusing screen.



ITZEROTT, G., & F. NIEMANN-Mikrophotographischer Atlas der Bakterienkunde. (Microphotographic Atlas of Bacteriology.) Leipzig, 1894, 8vo, xii. and 115 pp., 126 microphotographic figs. in 21 plates.

# (5) Microscopical Optics and Manipulation.

The Birefractometer or Eye-piece Comparer.\*—M. J. Amann describes the instrument which he uses for determining the double refraction of crystalline bodies which exhibit this property.

The birefractometer or eye-piece comparer consists principally of a wedge of selenite or of quartz carrying a micrometer division, at each division of which the thickness has been very exactly determined. This wedge can be inserted beneath the ocular lens of the Huyghens eycpiece, just as a micrometer, in such a way that its longitudinal axis makes with the principal sections of the nicols an angle of  $\pm 45^{\circ}$ .

\* Zeitschr. f. wiss. Mikr., xi. (1895) pp. 440-54.

The author explains carefully the method of calibrating the wedge, and gives the usual demonstration, according to Fresnel's theory, of the formulæ relating to the nature and intensity of the interference tints produced in doubly refracting bodies in parallel polarized light. For the ordinary conditions of work with the nicols crossed and the principal section of the wedge orientated at  $\pm 45^{\circ}$  to the plane of primitive polarization the intensity J of the ray from the analyser is given by

$$\mathbf{J} = \sin^2 \pi \frac{(\mathbf{N}_o - \mathbf{N}_e) \mathbf{D}}{\lambda},$$

where  $N_o$  and  $N_c$  are the indices of refraction of the doubly refracting body corresponding to the ordinary and extraordinary rays, D the thickness of the wedge at the given point, and  $\lambda$  the wave-length of the light.

The intensity of the coloration will be a maximum when

$$\left(\mathbf{N}_{o}-\mathbf{N}_{e}\right)\mathbf{D}=\left(2\,k+1\right)\frac{\lambda}{2},$$

where k is any whole number.

The tints given by the wedge between crossed nicols thus correspond to those of Newton's rings, since the rings which have the maximum intensity are those for which the relation holds:

$$2 e = (2 k + 1) \frac{\lambda}{2},$$

where e is the thickness of the layer of air.

Now, in the case of the wedge the difference of path  $\theta$  of the ordinary and extraordinary rays is given by

$$\theta = \frac{(\mathbf{N}_o - \mathbf{N}_e) \mathbf{D}}{\lambda}.$$

If, therefore, the wedge is examined in homogeneous polarized light of wave-length  $\lambda$ , it will present bright parts corresponding to thicknesses such that the difference of path  $\theta$  is equal to an uneven number of half wave-lengths, and dark parts where the thickness is such that the difference of path is equal to an even number of half wave-lengths, i. e. to a whole number of wave-lengths.

This gives a first means of calibrating the wedge, viz. by determining what is the difference of path of the two interfering rays and, therefore, the thickness for each point of the wedge.

In the case of the selenite, according to the determinations of Descloiseaux and Angström, in sodium light ( $\lambda = 0.589 \ \mu$ ),

for the axis of elasticity c :  $n_g = 1.5297$ ;

for the axis of elasticity  $a : n_p = 1.5206$ ;

whence  $n_g - n_p = 0.0091$ .

We have, therefore, for the first dark band

$$0.0091 D_1 = 0.589 \mu;$$

for the second,

 $0.0091 D_2 = 1.178 \mu$ 

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In order to dispense with the use of homogeneous light, the author prefers to the above method one, no less exact, depending on the observation of the bands of Müller, which appear in the spectrum when the interference colours presented by doubly refracting plates in polarized light are analysed by the spectroscope. In this method, the wedge of selenite is placed, as before, between the crossed nicols with its axes orientated at 45° to the principal sections of the nicols. Above the analyser, which is directly above the objective, is placed a direct-vision spectroscope provided with a special apparatus for determining the wavelength in the different parts of the spectrum. The lateral displacement of the wedge in the azimuth 45° is effected by a micrometer screw, and can be measured to 0.05 mm, nearly. In these conditions the dark bands in the spectrum are observed and their position accurately determined by means of the scale of the micro-spectroscope. The position and number of these bands varies with the nature and thickness of the double-refracting plate.

With daylight instead of homogeneous light the interference colour is composed of all the colours for which the difference of path  $\theta$  of the two interfering rays is equal to

$$\theta = (2k+1)\frac{\lambda}{2},$$

while in this tint there are wanting all the colours for which

$$\theta = 2\,k\,\frac{\lambda}{2}.$$

The parts of the spectrum corresponding to these absent colours are therefore dark, and form the bands of Müller.

Denoting by  $\delta$  the constant difference  $N_e - N_o$ , we have then for the first dark band corresponding to the wave-length  $\lambda$ 

$$\delta D_1 = \lambda,$$

which gives the thickness of the wedge at the point considered, and similarly for the second, third . . . . bands.

Having determined the thickness of the wedge at each point of the micrometer division, the tints of the Newton rings which correspond to these thicknesses are given by

$$2e = D(N_o - N_o),$$

i. e. for the selenite 2e = 0.0091 D, where e is the thickness of the layer

of air corresponding to the ring considered. The exact determination of the position of the bands of Müller in the spectrum offers some difficulty owing to their considerable width when thin layers are observed. For this reason 6 to 10 observations must be made and the mean taken. The bands are so much narrower, the thicker the section. This leads to a slight modification of the method of calibration described. This modification consists in superposing the wedge on a doubly refracting plate of the same nature as that of the wedge and of such a thickness that it takes the white colour between crossed nicols and gives a certain number (4 to 6) of the bands of Müller throughout the length of the spectrum.

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The exact position of these bands is determined once for all, and the displacements which they undergo when the wedge is superposed, first so as to augment the effect of the plate, and secondly so as to subtract from it.

The difference of path of the system will be in the first case

in the second,

$$\Theta = \theta + \theta',$$
$$\Theta = \theta - \theta',$$

 $\theta'$  being the constant difference produced by the plate,  $\theta$  the variable one caused by the wedge. But we have

$$\theta' = \mathbf{D}' \delta$$
,

where D' is the known thickness of the plate, and

$$\theta = D \delta$$
.

Therefore,

$$\mathbf{D} = \frac{\boldsymbol{\Theta} \ \boldsymbol{\mp} \ \boldsymbol{\theta}'}{\boldsymbol{\delta}} \boldsymbol{\cdot}$$

The author's determinations of the thickness at different points of the ordinary wedges offered for sale showed how very irregular was their form.

The method of using the birefractometer is very simple and convenient. The crystal section under examination is orientated so as to give the maximum brightness between crossed nicols, and the birefractometer is then slid in beneath the analyser until the doubly refracting body is obscured as completely as possible. When this happens, the difference of path produced by the crystal is exactly compensated by that caused by the wedge. The division on the latter at which the compensation takes place is then noted, and thus from the table giving the constants which accompanies each instrument, the exact value of the interference tint exhibited by the crystal, in the thickness of the layer of air of the Newton's rings corresponding to this tint, can be determined.

## β. Technique.\*

### (1) Collecting Objects, including Culture Processes.

Ilosvay's Reagent as a Test for Cholera Bacilli and other Bacteria.<sup>†</sup> — Dr. M. Lunkewicz finds that Ilosvay's reagent is an extremely sensitive test for the nitrous acid produced by cholera bacilli and some other organisms. The reagent is composed of two fluids kept apart and mixed in equal volumes when required for use :—I. Naphthylamin 0.1; aq. destill. 20.0; acid. acetic. dil. 150.0. II. Acid. sulphanilici 0.5; acid. acetic. dil. 150.0. The naphthylamin is boiled with water, and, after standing, the supernatant clear fluid is decanted off, and then the acetic acid added.

To bouillon or pepton cultures about one-fifth of their bulk of the

<sup>\*</sup> This subdivision contains (1) Collecting Objects, including Culture Processes; (2) Preparing Objects; (3) Cutting, including Imbedding and Microtomes;
(4) Staining and Injecting; (5) Mounting, including slides, preservative fluids, &c.;
(6) Miscellaneous. + Centralbl. f. Bakteriol. u. Parasitenk., xvi. (1894) pp. 945-9.

reagent is added. In a few seconds a red colour appears. The hue varies somewhat with the nutritive medium. The reagent may be used for solid as well as for liquid media, though the results with agar are less effective and much slower in developing than with gelatin.

For using this test two points must be borne in mind :--(1) that some commercial peptons will often give a reaction, because they contain nitrous acid (the "peptonum siecum ex albumine" is free from this objection); (2) fluid gelatin will also give the reaction. Hence the cultures should be laid in ice before applying the reagent, which also should be cold. In this way confusion between the colour from the organisms and that of the medium is avoided.

The reagent was tried with more than 30 bacteria, Vibrio choleræ asiaticæ giving the most characteristic reaction. With V. Metchnikovi the reaction was weaker, and with other vibrios of Finkler-Prior, Müller, Deneke there was none at all. The author states that Bacterium coli commune gives the reaction, but adds in a note that sometimes there was failure. There was no reaction with B. typhi abdominalis and many others.

In conclusion, the author points out that this nitrous acid reaction is superior to the indol reaction in that it can be obtained in a shorter time, that it can be used for pepton and gelatin cultures, that the colour developed is more intense, and that it can distinguish between cholera bacilli on the one hand and the Finkler-Prior and Müller's bacteria on the other.

Hens' Eggs as a Cultivation Medium for the Cholera Vibrio.\*— Drs. R. Abel and A. Dräer have investigated the suitability of hens' eggs for growing the cholera vibrio. The material worked with was obtained from cholera fæces, from intestinal cholera corpses, and from old cultivations. From each source four eggs were inoculated and incubated at  $37^{\circ}$ . On the 8th, 14th, 18th and 21st days one egg from each of these stocks was examined especially for H<sub>2</sub>S, and for alterations of colour and consistence. Microscopical preparations were made, and also cultures in pepton water and bouillon, on gelatin plates, on agar, and also on agar by the pyrogallic acid method.

Direct egg to egg inoculation was also tried in order to ascertain if any micro-organisms had been overlooked owing to their small numbers. The authors concluded that the hen's egg is a very unsuitable cultivation medium; that the cholera vibrio is present as a pure cultivation, both when the yolk is of golden-yellow colour and when it is greenish black. The explanation of these facts seems to be due on the one hand to the impurities which naturally exist in the egg, and on the other to differences in the derivation of the vibrio, some having considerable power of blackening the yolk, others very little, and again, the number of organisms introduced appears to have a certain influence. It was further found that the cholera vibrios developed  $H_2S$  in some eggs, and in others not.

Plaster and Brick Blocks for growing Yeast.<sup>†</sup>-M. J. C. Nielsen contests the statement made by Wichmann<sup>‡</sup> relative to the blocks for

<sup>\*</sup> Centralbl. f. Bakteriol. u. Parasitenk., 1º Abt., xvii. (1895) pp. 85-7.

<sup>+</sup> C.R. Travaux Laboratoire de Carlsberg, iii. (1894) p. 179.

<sup>‡</sup> See this Journal, 1894, p. 127.

growing yeast. The latter recommended that these cubical blocks should be made of firebrick instead of plaster of Paris. The author agrees with Klöcker, who found that spore formation, like all other physiological functions, was subject to oscillations, determined partly by the condition of the cells themselves, but also by slight variations in the experiments. Now these oscillations are less marked with plaster blocks than with those made of clay, and hence the plaster blocks are superior.

Vitreous Humour as a Nutrient Medium.\*-Dr. Herrnheiser has tested the value of vitreous humour as a nutrient medium, and it seems to answer very well. About 10 ccm. of vitreous is obtained from each eye (bullock) by incising the sclerotic. The fluid obtained is at once steam sterilized for half an hour, then filtered and afterwards sterilized again. The fluid, after these procedures, is somewhat milky from coagulation of the albumen. Besides using the fluid thus obtained, the author also employed modifications, rendered advisable for experimental purposes, owing to the richness in saline substances and to the poverty in proteid, by diluting with water and by adding 1 per cent. pepton solution. Comparative experiments with these three varieties of vitreous medium and also control cultivations with bouillon and sugar bouillon showed that the undiluted non-peptonized vitreous was the most effective medium for most bacteria.

Production of Sporeless Anthrax.<sup>+</sup>-MM. H. Surmont and E. Arnould have re-tried the methods for obtaining sporeless anthrax, and they infer from their experiments that it is very difficult to transform certain races of anthrax into sporeless ones. The best method for attaining a positive result is that of Roux, and if the results be not immediate it becomes easy to succeed by previously heating the cultures up to 42°, sowing them again every five days. The methods tried were four in number.

(1) Roux's method, where carbolic acid is added to the bouillon. The bouillon was alkaline veal broth, to which carbolic acid in the proportion of 1-10,000 to 20-10,000 was added. After sterilization the tubes were inoculated with the blood of a guinea-pig dead of anthrax. The presence or absence of spores was determined by placing bouillon cultures in water heated to 65° for 15 minutes.

(2) Method of Chamberland and Roux; this consists in adding bichromate of potash to the medium 1, 2, and 3 per 4000.

(3) Addition of hydrochloric and rosolic acids to the medium or Behring's method. Neither of these reagents was successful.

(4) Application of heat or the method of Phisalix. Pasteur's flasks containing bouillon inoculated with anthrax were incubated at 42°, and resown every five days.

New Method of Preparing Culture-media.<sup>‡</sup>-Dr. Lorrain Smith describes a method for preparing media suitable for the cultivation of The principle of the method consists in the addition of a bacteria.

\* Prager Med. Wochenschr., 1894, Nos. 22, 24. See Centralbl. f. Bakteriol. u. Parasitenk., xvi. (1894) pp. 980-1.

† Ann. Inst. Pasteur, viii. (1894) pp. 817-32. ‡ Proc. Cambridge Phil. Soc., viii. (1894) p. 217.

small percentage of alkali to fluids which contain proteids, such as white of egg and the serum of blood. The fluid is then heated to the boiling point or over it in the autoclave. By this means it is converted into a clear transparent jelly. It is then a medium suitable for the growth of a large variety of germs.

Preparation and Properties of Tetanus Antitoxin.\*—Dr. R. T. Hewlett prepares tetanus antitoxin by cultivating the bacillus in yeast flasks in an atmosphere of hydrogen, the medium used being grape-sugar bouillon. After incubating at  $37^{\circ}$  for three to four weeks the cultures to which carbolic acid is added in the proportion of 0.5 per cent., are filtered through a Chamberland bougie. One-hundredth of a ccm. of the toxin thus obtained will kill a guinea-pig weighing 300-400 grm.

The toxin is next weakened by adding an equal volume of Gram's iodine solution, and this mixture is injected subcutaneously into a horse, starting with  $\cdot 5$  ccm. and going up 22 ccm. After this intravascular injections were made; these ranged from 4–70 ccm. and were continued for about 5 weeks, when a period of a month without injections was allowed to elapse. At the end of this time the antitoxic power of the serum was found to be about 50,000. After this the treatment was rapidly pushed until the antitoxic power was equal to 1,000,000.

The antitoxic serum was obtained by bleeding from the jugular vein into sterilized vessels, and after 24 hours pipetting off the clear serum used for injection. To obtain the substance dry, it must be evaporated in vacuo over sulphuric acid. According to the author the experimental effects of the antitoxin smack of the marvellous; thus 0.0005 ccm. will protect a 400-500 grm. guinea-pig from 0.01 ccm. of the toxin, and a mixture of the two in the proportion of 50 to 1 is quite inert.

Alkali Albuminates in the Preparation of Cultivation Media.<sup>†</sup> — Dr. G. Deycke <sup>‡</sup> prepares agar for isolating diphtheria bacilli in the following way:—1 per cent. alkali albuminate, 1 per cent. pepton, 1/2 per cent. salt, 2 per cent. agar, and 5 per cent. glycerin are mixed with the adequate quantity of distilled water. This mixture is first neutralized with pure hydrochloric acid and next alkalinized with 1 per cent. of a soda solution consisting of 1 part soda and 2 parts water. After standing for several hours at room temperature, the mixture is boiled in a sterilizer for 3/4—1 hour. The hot agar is then filtered through a thin layer of cotton-wool into test-tubes, and then steamsterilized for 1/2 hour, after which it is allowed to set in oblique position. Prepared in this way the agar is a little cloudy, but the procedure has the advantage of rapidity.

For cultivating cholera bacilli a nutrient gelatin is made thus:—  $2\frac{1}{2}$  per cent. alkali albuminate, 1 per cent. pepton, 1 per cent. salt, and 10 per cent. gelatin are mixed with the proper proportion of distilled water and then neutralized with 2 per cent. of the before-mentioned soda solution. After this it is boiled for  $1\frac{1}{2}$ -2 hours in a steamer, and finally filtered in a hot-water funnel through blotting paper into test-tubes, which are boiled for 10 minutes on three consecutive days.

<sup>\*</sup> Brit. Med. Journ., March 2, 1895, pp. 464-5.

<sup>+</sup> Centralbl. f. Bakteriol. u. Parasitenk., 1º Abt., xvii. (1895) pp. 241-5.

<sup>‡</sup> See also this Journal, 1894, p. 750.

Even better than the preceding is a mixture of gelatin and agar, for it combines the advantages of both, and can be incubated at  $37^{\circ}$  without liquefaction:—2 per cent. agar, 5 per cent. gelatin,  $2\frac{1}{2}$  alkali albuminate, 1 per cent. salt, and 1 per cent. pepton, with the necessary quantity of water, are gently heated until the constituents are dissolved. After neutralizing with 2 per cent. of the soda solution the mixture is boiled for two hours in a steam sterilizer, and then filtered through a thin layer of cotton-wool into capsules.

Antiseptic and Disinfecting Properties of Formic Aldehyde.<sup>\*</sup>— M. H. Pottevin who has made experiments as to the antiseptic property of formol, finds that the number of germs sown in a medium to which formol has been added has a definite influence on the result, i.e. the prevention of growth, and also the time required for killing the germs. The action of the disinfectant is aided by moisture and elevation of temperature. Formic aldehyde has also a direct action on diastase, the activity of which is diminished or suppressed. Injected into animals formol exhibits irritant properties as shown by the necrosis of the tissues, but its toxicity is relatively small. As the vapours given off by saturated solutions and by the powder of trioxymethylen are noxious, disinfection with formic aldehyde requires great caution.

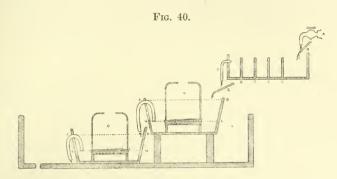
Dr. P. Miquel discusses the disinfectant properties of formic aldehyde from the practical aspect. By mixing an aqueous solution of commercial formic aldehyde (probably a paraldehyde or a mixture of formic aldehydes in different conditions of polymerism) of a density of 1.07 to 1.08 with calcium chloride, so that the resulting liquid has a density of 1.20, a fluid is obtained with which linen cloths can be soaked; when hung up these cloths give off the vapour of formic aldehyde very copiously, and if placed in a confined space serve to disinfect very actively. For loose articles such as books, bedding, &c., this method acts well, but for the disinfection of rooms and large spaces it is not applicable.

Apparatus for the Care and Hatching of Fresh-water Fish Eggs.†—Mr. A. T. Holbrook describes an arrangement which for laboratory and experimental use has been found to answer all ordinary requirements. In addition to the apparatus, nothing is needed except a steady supply of tolerably clean cold water, a cool clean room and a sink or water escape. The water enters at the faucet A (fig. 40) and splashes on the board B; it is thus aerated and falls into a box containing a number of filtering partitions. This box is water-tight and provided with a set of four flannel filters of different grades of fineness. All use of metal in the apparatus is avoided, except where necessary, and then it is covered with paraffin or other harmless substance. The water leaves the filter-box by means of a tube near the top, from which it falls on to another board E, being thus further aerated as it falls into a rather large dish or trough ; placed in this trough is a bell-jar N, with a considerable opening at the top, and with a piece of fine netting stretched and held in place across the bottom of the jar by a rubber At the further end of the trough is placed a siphon F. When band.

\* Ann. Inst. Pasteur, viii. (1894) pp. 796-810; Ann. de Micrographie, vi. (1894) pp. 588-95. † Bull. Mus. Comp. Zool., xxv. (1894) pp. 93 and 4.

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the apparatus is in running order the eggs are placed in the bell-jar and spread upon the cloth in a single layer. The short end of the siphon is placed in the trough in such a position that when the water is siphoned off, the eggs will not be left out of water. The apparatus so works that



the water alternately rises and falls, washing the eggs constantly in a gentle way, but not heaping them together, nor disturbing them to any considerable extent. The water that is siphoned from the first trough falls into another smaller trough, supplied with jar and eggs, and placed far enough below the first trough to prevent interference with the siphoning.

RODET, A.—Sur la stérilisation du lait. (On the Sterilization of Milk.) Rev. d'Hygiène et de Police Sanitaire, XVI. p. 1025.

#### (2) Preparing Objects.

Preparation of Fish Embryos.\*-Mr. A. T. Holbrook points out that Henneguy's method of treating eggs with Müller's fluid has the objection that the yolk is dissolved out in such a manner as to be subsequently precipitated around the eggs, which are thus left imbedded in the precipitated mass. This difficulty may be overcome by cutting the egg-membrane in such a way as to catch an air-bubble within the eggshell, and thus to float the egg in the fluid. The author reports that his best results were secured with material killed in picro-sulphuric acid. The embryos were left for three hours in the fluid, and then removed and dehydrated by passing them slowly through successively stronger grades of alcohol. In staining, the eggs were passed through weaker grades of alcohol and then stained for 24 hours in Czokor's cochineal. Mr. Holbrook found it best to cut his eggs without attempting to remove the membrane or any of the hardened yolk. Great difficulty was experienced in making sections, as parts of the yolk break up and fall out of the section, often carrying the embryo with them, while the firm membrane is apt to resist the knife, and fold or break the embryo. It was found to be a good plan to have a generous amount of imbedding substance about the object and to cause the knife to strike the embryo before the yolk, as there is thus less danger that the embryo will be turned or pushed by the resisting yolk.

\* Bull. Mus. Comp. Zool., xxv. (1894) pp. 82 and 3.

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Examining Blood of Vertebrata for Endoglobular Parasites.\* -M. A. Labbé finds that methylen-blue is the most useful reagent for examining fresh specimens of parasitic Protozoa, and gives the following formula :- Methylen-blue, 1; H<sub>2</sub>O, 100; NaCl, 0.75. A drop of this fluid is placed on the slide near the cover-glass, under which it is drawn by suction with a piece of blotting-paper. Acetic carmine and 1 per cent. methyl-green are also favourably spoken of. The best fixatives are sublimate, osmic acid, a mixture of equal parts of ether and absolute alcohol, picric acid and Flemming's fluid.

The procedure adopted by the author is first to pass the cover-glass through the flame and wash in distilled water for 5 minutes. When dry, immerse in acetic acid, 1 drop; H<sub>2</sub>O, 20 ccm.; after this it is treated for 24 hours with saturated aqueous solution of pieric acid, 30;  $H_2O$ , 30; glacial acetic acid, 1. It is next washed in absolute alcohol.

The staining methods recommended are—(1) Malachowsky's. Saturated aqueous sol. methylen-blue, 24; 5 per cent. sol. borax, 16; H<sub>2</sub>O, 40. With this the preparations are treated for 24 hours and then washed. (2) Czenzinski's. (a) Saturated aqueous solution methylen-blue, 2;  $H_2O$ , 4. (b) 1 per cent. solution of eosin in 60° spirit. Mix 1 part of (b) with 2 of (a), and stain for 24 hours. (3) Mannaberg's. Saturated aqueous solution methylen-blue, 40; 2 per cent. eosin in C<sub>2</sub>H<sub>6</sub>O at 60°, 80; H<sub>2</sub>O, 40. (4) Hæmatoxylin. (a) Hæmatoxylin solution, 10;  $C_2H_6O$ , 100. (b) 1/2 per cent. solution ammonia-alum; 1 part of (a) and 2 of (b). Stain for 24 hours. Then differentiate in nitric acid alcohol, followed by ammonia-alcohol. Wash in 80 per cent. spirit. Balsam.

Besides the preceding numerous hints are scattered throughout the author's exhaustive paper.

For removing the pigments from Gymnosporidia the author used the following:-Glycerin, 100; alcohol 70 per cent., 100; a few drops of HCl.

Preparation and Care of Insect Collections.<sup>†</sup>-Mr. C. Dury has prepared an essay on the best methods of killing, pinning, mounting, and taking care of material as well as on the proper methods for collecting It will probably be found useful by those whose experiences are not it. sufficient to enable them to do this for themselves.

Karyokinesis of Spirogyra.<sup>‡</sup>—Dr. J. W. Moll fixed the filaments in Flemming's mixture for four days. Fragments were placed in a 6 per cent. solution of celloidin (dissolved in equal parts of ether and 90 per cent. alcohol). The addition of a minute quantity of gentianviolet made the recognition of the objects easier. After a few minutes they were placed on a slide with a drop of celloidin, and the slide placed in 95 or 96 per cent. alcohol. The hardened layer of celloidin was cut into square centimetres; the squares were lifted off the slide and placed in 96 per cent. for  $1\frac{1}{2}$  hours. The alcohol was replaced with oil of marjoram; this requires to be done carefully. Then the objects were transferred through solutions of paraffin and oil of marjoram (15, 30,

<sup>\*</sup> Arch. Zool. Exp. et Gén., ii. (1894) pp. 55-258 (10 plates).

Journ. Cincinnati Soc. Nat. Hist., xvii. (1894) pp. 173-80.
 Arch. Néer. Sci. Nat., xxviii. (1894) pp. 312-57 (2 pls.). Cf. this Journal, 1893, p. 752.

45, 60, 75, and 90 per cent.); and finally cut. We have said enough to show that Dr. Moll goes in for somewhat elaborate technique, but he has much more to say on this subject.

Cell-structure.\*-Herr G. Schloter, in studying the cells of the Salamander (skin and liver), used corrosive sublimate as fixative, and stained with Böhmer's hæmatoxylin+indulin+eosin+safranin, or with Böhmer's hæmatoxylin + aurantia, or with Ehrlich's stains.

# (3) Cutting, including Imbedding and Microtomes.

Anise Oil in Histological Technique.<sup>†</sup>—Dr. V. A. Moore has obtained very satisfactory results from a modification of Kühne's ‡ method of imbedding in anise oil. The pieces to be sectioned should be from 2-4 mm. thick. These are placed in a test-tube, on the bottom of which is some cotton-wool and which contains absolute alcohol. The tubes are heated in a water-bath to about 40° for half-an-hour or so, by which time the tissue is sufficiently hardened. The blocks are then mopped with blotting paper, and having been covered with anise oil sectioned on a freezing microtome. In this way specimens can be examined in less than an hour after removal from the body, a point of considerable importance very often.

By previously staining the tissue en masse much time is saved if several or many sections from the same block are intended to be kept. This is rendered possible by the fact that anise oil and Canada balsam are miscible; consequently, the sections can be transferred directly from the knife to the mounting medium, provided the block has been thoroughly impregnated with the oil.

#### (4) Staining and Injecting.

Staining Attraction-spheres and Centrosomes.§-Mr. J. H. Schaffner recommends the following two processes for observing the attraction spheres and centrosomes in vegetable cells:—(1) Fix the objects for 1 or 2 days in a solution of 15 parts 1 per cent. platinum chloride, 1 part acetic acid, 2-4 parts 2 per cent. osmic acid, 80 parts water. Now wash the objects in flowing water, harden gradually in alcohol, and after that place them from 12 to 18 hours in pyroligneous acid. Next place them in a solution of 1 part 20 per cent. hæmatoxylin and 99 parts 70 per cent. alcohol. Keep in the dark, and leave from 12 to 18 hours, and after that in the dark for some time in 70 per cent. alcohol. Imbed and section. After the sections are fastened to the slide, cover them with a solution of potassium permanganate of a light rose colour, and leave until they have an ochre colour. Then wash in a solution of 1 part hydric oxalate, 1 part potassic sulphate, and 1000-2000 parts water. After this stain the sections from 3-5 minutes in a saturated alcoholic solution of safranin; clear, and mount in Canada balsam. The centrosomes are stained very black by the safranin; while the attrac-tion-spheres remain quite colourless or are only very slightly stained. (2) Place the sections for 30-35 minutes in a 1 per cent. aqueous solu-

<sup>\*</sup> Arch. f. Mikr. Anat., xliv. (1894) pp. 249-59 (1 pl.).

<sup>†</sup> Amer. Mon. Micr. Journ., xv. (1894) pp. 373-6.
‡ See this Journal, 1892, p. 706.
§ Bot. Gazette, xix. (1894) pp. 451-3.

tion of ferrous sulphate, then wash, place for the same length of time in a 5 per cent. aqueous solution of tannin, wash again, again cover with the iron solution, and leave for a minute or two, or until they change to a rather dark colour. After washing off the iron, stain with anilinsafranin (1 part 1 per cent. alcoholic solution of safranin and 2 parts water) from 30 minutes to 1 hour, and then 15 minutes or more in an aqueous solution of picro-nigrosin long enough to take a dark bluishgreen colour. Then raise through the grades of alcohol, and mount in Canada balsam. The centrosomes are by this process stained very dark; -and the attraction-spheres are well defined, often showing the radiate structure.

Flagella-Staining.\*-Dr. R. Bunge has improved his method of staining flagella in the following manner.† After filtering a requisite quantity of the mordant, which should be a few days old, into a testtube, peroxide of hydrogen is added, drop by drop, until the mordant becomes of a red-brown hue. It is then shaken up and filtered over the prepared cover-glass, on which it is allowed to act for about 1 minute. The cover-glass is then mopped up, dried, and stained, preferably with carbol-gentian. The flagella seem swollen and are so deeply stained that this can never be overlooked. Besides staining the flagella the capsules are also simultaneously coloured by this method.

Lithium Bichromate as a new Reagent for Hardening Adult Brains.<sup>‡</sup>—Mr. O. S. Strong reports on the use of this reagent in the application of Golgi's method. As is well known, the principal defects of this method are the uncertainty of good results, inequality of the impregnation, and the expense in the use of so much osmic acid. It occurred to Mr. Strong that the defects could be remedied to some extent by enhancing the penetration of the silver nitrate. This was attained by mixing the silver nitrate solution with solutions of sodium or zinc sulphate. In certain cases this modification gave very good results, but further experience is necessary to determine its exact value. Lithium bichromate, however, promises to prove valuable in certain lines of work. The long Golgi's method dispenses with the osmic acid, and therefore with the expense, but the period of time is so long that it would be an advantage to be able to shorten it. This time may be reduced from the 20 or 30 days now required to 1 to 3 days, by simply using lithium bichromate instead of potassium bichromate. The tissue should be cut into small pieces, and put into a liberal supply of a 2 to 3 per cent. solution of lithium bichromate. In the course of 1 to 2 days pieces should be placed in the silver nitrate solution at intervals. A piece found to be impregnated may be washed with strong alcohol for about half-an-hour, stuck on a microtome block, and cut in 95 per cent. alcohol into sections of suitable thickness. After being washed in 95 per cent. alcohol, these may be cleared in Ol. origanum cretici, rinsed in xylol and mounted in dammar-balsam without a cover-slip. For the human brain and for that of the hen, it has been found that this method

\* Fortschr. d. Med., xii. (1894) No. 24. See Centralbl. f. Bakteriol. u. Parasitenk., 1° Abt., xvii. (1895) pp. 102–3.
† See this Journal, 1894, p. 640 ; 1895, p. 129.
‡ Trans. New York Acad. Sci., xiii. (1894) pp. 237–9.

gives good results, but it appears to be inferior to the ordinary rapid method for embryonic material.

JOHNE-Zur Färbung der Milzbrandbacillen. (Staining Baeilli of Anthrax.) Dtsche. Tierärztl. Wochenschr., 1894, No. 35, pp. 289-92. Dtsche. Zeitschr. f. Tiermed., 1894, XX. Nos. 5/6, pp. 426-9.

### (5) Mounting, including Slides, Preservative Fluids, &c.

Wiese's Preserving Fluid.\*-Dr. A. B. Meyer reports on his experience with Wiese's fluid (6 gr. sodium hyposulphite in 5000 gr. water, 75 gr. ammonium chloride in 250 gr. of water, mixed with 4-6 litres of spirit). It certainly allows the colours of fishes, reptiles, &c., to remain, but unfortunately it does not prevent decomposition, as Meyer recently experienced with some tropical fishes thus preserved. The much desired fluid is still to seek.

Formalin as a Preserving Medium for Vegetable Tissues.<sup>+</sup>-Prof. O. Penzig recommends the use of formalin or formol-a solution of 40 parts formaldehyd in 100 of water-for the preservation of vegetable substances. A mixture of this solution in water to the extent of 2-2.5per cent. has powerful antiseptic properties. The colours of chlorophyll and anthocyan are, to a certain extent, also preserved.

Herr E. Bruns ± speaks favourably of the same substance as a liquid for the preservation of green, red, and brown seaweeds, using a 1 per cent. solution of formalin in sea water. The colour of the algæ is perfectly preserved, if protected from light.

Dr. L. Linsbauer § also recommends formol as a preservative of the delicate parts of plants, either in the form of vapour, or as a 2.5 per cent. solution in water. It prevents shrinking in the objects immersed; some colours of flowers are well preserved.

# (6) Miscellaneous.

Detection of Phosphorus in Vegetable Tissues. - Dr. G. Pollacci recommends the following method. The phosphorus is first brought into the state of ammonium phospho-molybdate by the application of a mixture of ammonium molybdate and nitric acid. The addition of stannous chloride SnCl<sub>2</sub> then produces a strong coloration, varying from dark blue to grey, according to the amount of phosphorus contained in the preparation.

Slide-holder.¶-Dr. L. Heim describes a stand which he finds useful for heating slides when being stained. The stand consists of a base, an upright, and a tray. The tray is connected with the upright by means of a horizontal piece which is fixed for vertical and also horizontal movement by two joints. The slide-holder is nothing more than the stand used by chemists for heating liquids over a flame.

- \* Zool. Anzeig., xvii. (1894) pp. 446-7.

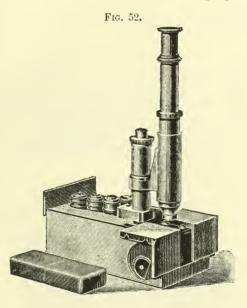
- \* Malpighia, viii. (1894) pp. 331-6.
  \* Ber. Deutsch. Bot. Gesell., xii. (1894) p. 185.
  \* SB. K. K. Zool.-Bot. Gesell. Wien, 1894, pp. 23-6.
- Malpighia, viii. (1894) p. 363.
   Centralbl. f. Bakteriol. u. Parasitenk., 1º Abt., xvii. (1895) p. 84 (1 fig.).

# MICROSCOPY.

#### a. Instruments, Accessories, &c.\*

(1) Stands.

Portable Microscope by Nachet & Sons.—Mr. E. E. Hill writes, "This compound Microscope is probably the smallest and most compact instrument in existence. It has many advantages over similar instruments, although it has some defects. The instrument is made throughout of brass, and when enclosed within its case measures  $3\frac{1}{2}$  by 2 by  $1\frac{1}{4}$  in, and weighs 15 oz.; when the draw-tube is pulled out to its fullest extent, a tube-length of  $4\frac{1}{2}$  in. is obtained. It has one Huyghenian eye-piece and four object-glasses, all mounted specially short. The stage is formed by the end of the brass case and a portion of the top springing open as



soon as the portion to which the limb of the instrument is attached is pulled away to open the case. The limb of the instrument slides on a grooved fitting and is retained in position by a small clamping screw. The fine-adjustment is of the direct spring form and is brought into operation by means of the milled head. The distance of the optic axis from the limb of the instrument is 3/4 in.; this is a defect for working with 3 in. by 1 in. slips, but for field and pond work a series of 6 slips 2 in. by 3/4 in. (3 with hollow and 3 plain) are supplied and packed into a small leather-covered case which packs inside the brass case. The

\* This subdivision contains (1) Stands; (2) Eye-pieces and Objectives; (3) Illuminating and other Apparatus; (4) Photomicrography; (5) Microscopical Optics and Manipulation; (6) Miscellaneous.

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concave mirror is enclosed in the case and is moved by means of the small brass milled head outside the case. This Microscope is supposed to have been made about 30 years ago by Messrs. Nachet and Sons of Paris for Dr. Mouat, for use in India.

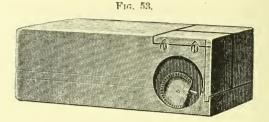
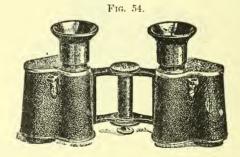


Fig. 52 shows the instrument arranged for use. Fig. 53 shows the instrument packed for carrying.

# (2) Eye-pieces and Objectives.

The new Zeiss Patent Binoculars.\*—Mr. E. M. Nelson gives an account of these new instruments. There are two kinds: one corresponding to the binocular field-glass (fig. 54) and the other to the binocular telescope (fig. 55). They are both astronomical telescopes consisting of an object-glass and a Huyghenian eye-piece, and the erection of the image is effected by two prisms. In the case of the simple telescope there is probably a greater loss of light by this prism

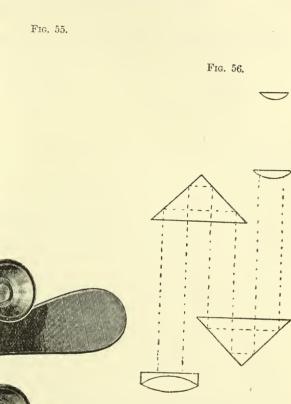


arrangement than with the ordinary erecting lenses, but in the case of the field-glasses several important points are gained by discarding the Galilean principle. Thus the large field and even definition of the Huyghenian eye-piece are secured. The most striking feature of the new instrument is that, to secure portability and increase the stereoscopic projection, the axis of the object-glass is not in the axis of the eye-piece. The author's notion of the arrangement of the object-glass, prisms and eye-piece, is seen in fig. 56; the rays are bent back twice, so that the instrument is reduced to about 1/3 in length.

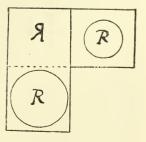
\* English Mechanic, lx. (1894) pp. 344-5.

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D.R.P.







The result of the following comparison between the new Zeiss fieldglass of 6 power and an old Galilean binocular of 7 power shows that the Zeiss had the better definition, and the Galilean the more light.

7						New Zeiss.	Old Binoculars.
Power	• •		••	••	• •	6	1
Weight	• •					$12\frac{1}{2}$ oz.	14 <u>3</u> oz.
Length				• •		$3\frac{1}{4}$ in.	$8\frac{1}{2}$ in.
Diameter of field	d		•••			6° full	2° bare
Diameter of obj							$2\frac{1}{8}$ in.
Focus of objectiv	ve	••	••	••	••	$4\frac{1}{2}$ in.	9 in.

The new instruments are made of three powers, 4, 6, and 8, and the author considers that for field, tourist, and ordinary purposes they have no rival.

The stereo-telescopes are constructed on the same principle as the field-glasses, but in them the ray is not bent back on itself. They may be used in two ways. In the first position the bodies of the telescopes point to the right hand and left hand of the object which is to be observed (fig. 55), while the axes of the eye-pieces are directed towards it. The greatest stereoscopic effect is thus obtained, while the object can be observed from behind cover, such as a tree or post. In the second position the bodies of the telescopes point up to the zenith. Here there is less stereoscopic effect, but it is possible to look over a wall without being exposed. These instruments also are made of three powers, viz. 6, 8, and 10.

In these new instruments each eye-piece screws in and out by itself, so that each eye can be focused for itself.

Fig. 57 is a plan showing the prisms in their proper positions as looked at from the object-glass. The larger circle represents the objectglass, and the smaller the field-lens of the Huyghenian eye-piece. The letter R, drawn on the object-glass, is made to represent the rays from an erect image falling on the object-glass. When the rays are brought to a focus by the object-glass, the erect image will be inverted and transposed. But because of the action of the upper prism in fig. 54, the inversion is corrected, while the transposition is left. The second prism, i.e. the lower one in fig. 54, whose edge is at right angles to the first prism, corrects the transposition, while the corrected inversion remains unaltered.

Klein's Lens with Micrometer.\*—Herr F. Becke describes an arrangement for measuring the small interference figure which is obtained with the Czapski eye-piece by inserting an iris-diaphragm in the plane of the image. The author does not remove the Ramsden eyepiece and examine the image formed directly by the objective, but with an aplanatic lens—the Klein's lens—observes the image which is formed above the Ramsden eye-piece.

The apparatus consists of a cylindrical tube-support which slides with slight friction over the head of the Czapski eye-piece. In the upper part of the support is an adjustable aplanatic lens with magnification of 8 times, and an eye-piece micrometer (10 mm. divided into 100 parts) which can be adjusted by two projecting pins.

\* Tschermak's Mineral. u. Petrogr. Mittheil., xiv. (1894) pp. 375-8. See Zeitschr. f. wiss. Mikr., xi. (1895) pp. 500-1.

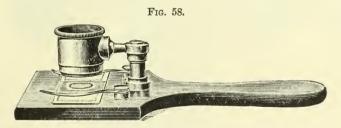
#### ZOOLOGY AND BOTANY, MICROSCOPY, ETC.

To make an observation with the Klein's lens, the part of the section under examination is first adjusted with the Czapski eye-piece; the irisdiaphragm of this eye-piece is then closed up so far that only the part of the section to be examined remains visible, and the Klein's lens is put on and adjusted so that the objective diaphragm is clearly visible; the interference picture is then plainly seen. Finally the scale is either raised or sunk until it also is sharply defined against the interference picture.

To determine the optic axial angle with this apparatus, the formula of Mallard D = M sin E must be used. In this formula E denotes the half optic axial angle in air, D the number of the scale divisions for E, and M a constant to be determined for the system by observation of a known optic axial angle. Instead of making use of the Mallard formula in each particular case, the author plots out a curve with the scale divisions as abscissæ, and the corresponding angles as ordinates.

Reichert Demonstration-Lens.\*—Dr. W. Behrens describes a lens lately brought out by the firm of Reichert, which is useful for showing microscopic preparations to a large audience, since the apparatus can be conveniently passed from hand to hand without disturbing the preparation.

It consists (as seen in the figure) of a black ebonite plate, with central aperture, on which the slide is held by means of two clips as



on a Microscope stage. The continuation of the plate behind forms a handle which is held in the hand during the observation of the preparation.

A short upright between the spring-clips forms the lens-holder. At the top it carries a short horizontal arm which can rotate about the vertical axis. This arm supports a spring socket in which slides the lens, so that it can be adjusted by hand on the preparation, while the apparatus is directed towards the sky or an artificial source of light.

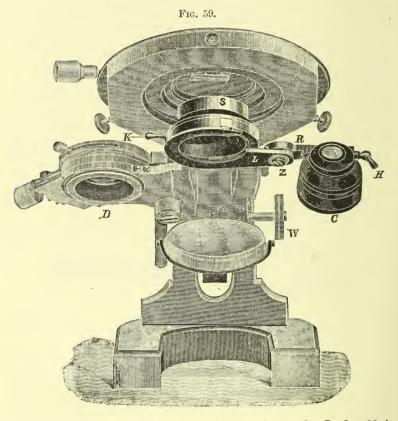
# (3) Illuminating and other Apparatus.

Illuminating Apparatus.<sup>†</sup>—Dr. S. Czapski remarks that the arrangements which have hitherto been proposed for the quick passage from convergent to parallel light, i.e. for the rapid removal of the condenser, either require a radical change in the stage and other parts of the Microscope (Fuess method), or have the disadvantage that the removal of the condenser is not momentary, since the whole illuminating apparatus

\* Zeitschr. f. wiss. Mikr., xi. (1895) pp. 458-9. + Tom. cit., pp. 433-40.

has to be lowered by rack-and-pinion motion before the condenser can be thrown out (Reichert method). Since, in working without condenser, cylinder diaphragms are generally necessary, these have also to be introduced. These operations require time and prevent the possibility of observing the immediate consequence of the change of illumination on the preparation.

The arrangement which the author has devised is free from these two disadvantages. It requires no reconstruction of the stand and no movement of the illuminating apparatus in the line of the optic axis.



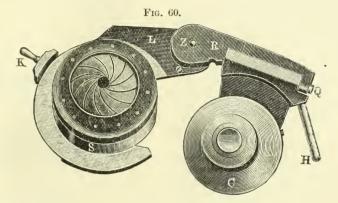
In fig. 59 the apparatus is seen as attached to the stand. In fig. 60 it is shown by itself as seen from above.

The socket S, of the size to fit into any spring socket of a Zeiss stand, carries on its lower flange a projecting piece L, which is bored through its other end for the reception of the pin Z. About this pin as vertical axis turns the frame R, to which is attached the condenser system C which is movable downwards to a stop about a horizontal axis Q. The movements about this axis and also about the vertical axis Z are both effected by the lever H.

# ZOOLOGY AND BOTANY, MICROSCOPY, ETC.

By the movement about the pin Z up to a stop, the frame R, with the condensor C, can be rotated from the position shown in the figure, until the latter is beneath the socket S, when a further pressure on the lever H serves to press the condenser up into the socket. The position of the condenser in which its axis coincides with that of the body-tube is fixed by a pin in the socket fitting into a small groove on the condenser. The diaphragm apparatus D is then brought beneath the condenser system in the usual way.

The removal of the condenser is effected by the same manipulations in the reverse order. The diaphragm apparatus D is swung to one side, the condenser C released from the socket by the lever H, and then turned aside out of the optic axis by rotation about the pin Z.



The apparatus can be provided with a cylinder diaphragm, whose use without the condenser is often desired although it may not be considered as necessary. This diaphragm, as seen in fig. 60, is an iris-cylinder diaphragm with curved lamellæ, so that it may be brought close to the preparation. When open it allows room for the condenser system, and even when the condenser is in position it can be closed slightly before touching it. When the condenser is removed, the diaphragm can be closed to a diameter of 0.5 mm. The movement of the diaphragm is effected by the knob K projecting from the lower flange of the socket S.

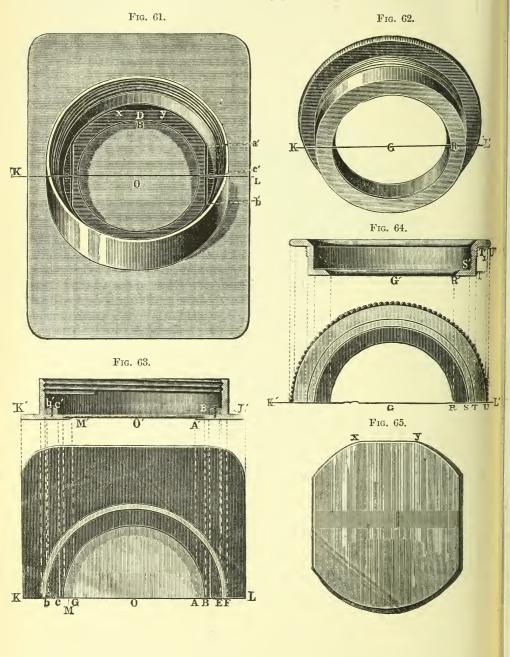
New Compressorium.\*—Prof. Fr. Sav. Monticelli describes a new and simple compressorium. It consists of two parts, a base plate and a ring, provided with a screw for applying the pressure.

The base plate is a rectangular brass plate with rounded angles (fig. 61) of dimensions  $80 \times 57 \times 1$  mm. In the middle of the plate rises a brass ring to a height of 9 mm. (fig. 63, E F), in thickness a little over 1/2 mm. and 47 mm. in diameter. On the inside this ring carries a screw-thread which does not occupy more than 5 mm. of the total height commencing from the upper margin. Within the ring the base-plate has a circular aperture 30 mm. in diameter. Round this aperture up to a diameter of 38 mm. the base-plate is cut through half

\* Zeitschr. f. wiss. Mikr., xi. (1895) pp. 454-7.

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its thickness, so that a circular step A B (figs. 61 and 63) is formed. On this step is fixed with mastic a circular glass slide of the same thickness as the step (figs. 61 and 63, O B). Between the glass slide and the contour of the ring rests a circular collar B E (fig. 63) of 4 mm. which carries in



relief two brass segments (a' b' c', fig. 61; b' c', fig. 63) opposite each other across the diameter KL (figs. 61 and 62). On the glass slide and circular collar rests the movable cover-glass, which is of the same thickness as the segments a' b' c' (figs. 61 and 63). In order that it may come in contact with the lower glass slide it is cut by two segments corresponding to the two which are on the circular collar. By this means the cover-glass can receive no movement of rotation. For convenience of removal it is cut across the top in the direction x y (figs. 61 and 65), normal to that of the segments.

The ring of compression (fig. 64)' is a ring of brass, of diameter a little less than that of the ring fixed to the base-plate. It is provided with a screw which engages in the screw-thread on the ring fixed to the base-plate. It has a diameter of 44 mm. and is provided at the top with a circular milled head (fig. 64, T U, T'U'), and below with a circular rim of 6 mm. (fig. 64, R S, R' S') which serves to augment the surface of compression on the cover-glass O (fig. 61).

The advantages claimed for the apparatus are the large field of view for microscopic observation, the condition of immobility of the coverglass, and the possibility, by means of the screw, of insensibly increasing or diminishing the pressure.

Compressorium.\*—Prof. H. E. Ziegler has made an improvement in the compressorium recently devised by him.† In the original apparatus only a small portion of the water passes beneath the cover-glass, for most of it flows between the caoutchouc ring and the lower glass plate. This is of little consequence when the stream of water is sufficiently strong, and the object is not too small; but if the object is so small that by the compression the space between the cover-glass and the lower glass plate is very narrow, the water beneath the cover-glass is almost completely at rest, and nearly all the water which flows through the apparatus takes its course between the caoutchouc ring and the lower glass plate.

The author has therefore added to the apparatus an arrangement by which all the water flowing through the apparatus must take its course beneath the cover-glass. For this purpose the caoutchouc ring at two opposite points is pressed in upon the lower glass plate by means of two pins, so that by the compression at these points there is no interval left between the ring and the plate. The space between the lower glass plate and the ring is thus divided into two parts; and the upper plate is so adjusted that the openings of the tubes conveying the water are brought over these parts.

#### (5) [Microscopical Optics and Manipulation.

Improved Method for the Microscopic Investigation of Crystals.t Mr. A. E. Tutton calls attention to an important memoir on the microscopic determination of crystals, contributed by Prof. Klein to the 'Sitzungsberichte' of the Berlin Akademie der Wissenschaften for January 31, 1895. In this communication a description is given of a new stage goniometer, which allows of the examination of many of the principal zones of a crystal with one and the same setting of the crystal on its

\* Zool. Anzeig., xvii. (1894) pp. 471-2., '† See this Journal, 1894, p. 759. † Nature, li. (1895) pp. 608-11. holder; it also contains an account of the method of examination of a crystal by immersing it in a liquid with refractive index the mean of the refractive indices of the crystal.

The Microscope employed (made by Fuess) is of the petrological type, with circular rotating stage provided with rectangular movements. The nicols are made to rotate simultaneously, as in the Allan-Dick Microscope made by Swift.

The stage goniometer is used with the Microscope in the horizontal position. The base-plate of the goniometer is clamped upon the now vertical stage. It carries a horizontal projecting piece, terminating in the supporting cone for the goniometer circle. The adjusting movements of the goniometer are simpler and lie closer together than in the ordinary form of goniometer. The glass cell containing the immersion liquid is supported by a stand with adjustable arm placed to the left of the Microscope.

The determination of the true optic axial angle 2 V can be made at once with the instrument if the crystal is immersed in a liquid with refractive index equal to the intermediate refractive index  $\beta$  of the crystal.

The advantage of the method consists in the fact that it is unnecessary to prepare sections of the crystal, for if the immersion liquid is properly chosen, the crystal will lose its outline and disappear in the liquid. For observation by this method, crystals should be chosen which are equally thick in two perpendicular directions in the plane of the optic axes, and not too thick to prevent the interference figures being observed.

Prof. Klein describes the method of determining with the apparatus the extinction angles upon the various faces of a zone, and shows how the method introduces a great simplification in the study of triclinic crystals, since the position of the optic axes can be at once determined.

Mr. Tutton shows what use he made of the method of observation in the course of his investigation of the sulphates of potassium, rubidium, and cæsium.\*

Indication of Magnification in Micrographic Drawings.<sup>+</sup>-Dr. D. Carazzi remarks that there are still naturalists who preserve the bad habit of not giving the indications of the magnification in the micrographic drawings which illustrate their memoirs, although nearly all are convinced of the necessity of so important an indication. The usual plan is to give with the drawing the number of the eye-piece and objective, the name of the maker, and the length of body-tube. Dr. De Vescovi 1 has pointed out that these three elements are not sufficient, and that account must also be taken of the real size of the object, and of that of the drawing obtained with the camera lucida. But as the operations involved in this method are long and troublesome, the former plan is still usually adopted. The author shows, however, how impractical and useless the method is, owing to there being no rational system of notation amongst Microscope makers. For this reason the name of the maker has to be given, and the reader has to refer to a number of makers' catalogues in order to understand the indication.

\* Journ. Chem. Soc., 1894, p. 628. † Zool. Anzeig., xviii. (1895) pp. 162-4. ‡ Op. cit., x. (1887) p. 197. For cases which require a rigorous exactness the author would keep the rule of noting the real magnitude of the object and that of the design projected by the camera lucida, but for ordinary purposes proposes a simpler method, which consists in placing on the stage the objective micrometer, and drawing its scale on the paper with the camera lucida. In this operation the eye must be kept at the same distance from the eye-piece, and the paper must be held near the stage and on a level with it.

The operation should be repeated with all the systems in use, and a table drawn up giving for each the number obtained by taking the ratio between the dimensions of the drawing and the real length of the divisions of the micrometer.

The indications of the table constructed in this way are not rigorously exact, but they represent mean values corresponding to the personal conditions of the observer, and give the magnification with sufficient approximation, so that they are certainly preferable to the pretended exactness of the data hitherto in use.

Resolving Power of the Microscope, and the Future of the Instrument.\*—M. J. Amann discusses the question of the limits to the resolving power of the Microscope. The theory of Abbe shows us that the formation of the image in the Microscope depends not only on the laws of geometrical optics, but also on the more complex phenomena of interference and diffraction. The microscopic structure beneath the objective determines the formation of a spectrum composed of a maximum corresponding to the luminous pencil not diffracted and of maxima of the second order. In the case of regular structures, the phenomenon is similar to that produced by gratings, and especially by gratings traced on transparent screens, which Quincke<sup>†</sup> has studied, and for which he has given the formula :

$$Jp^{2} = p^{2} \cdot 4\cos^{2}\left\{\frac{a}{\lambda}(n-1) + \frac{b\sin a}{\lambda}\right\} \pi \cdot \left(\frac{\sin^{2}\frac{p \cdot b\sin a}{\lambda}}{p^{2}\sin^{2}\frac{b\sin a}{\lambda}}\pi\right)^{2},$$

representing the distribution of the vibratory movement in the plane in which the diffractive spectrum is formed.

When the source of light is very distant, the diffraction spectra are formed in the focal plane of the objective. The final image which the Microscope gives us of such structures results from the interference of the rays emanating from the spectrum in the particular conditions of delimitation of the latter by the aperture of the objective. The mathematical expression of the repartition of the vibratory movement in the plane of the image takes the form of an integral of Fourrier. For the Microscope to give us an image of these structures it is necessary and sufficient that the objective admit besides the absolute maximum, at least one maximum of the second order in the case of a structure similar to a

\* Arch. Sci. Phys. et Nat., xxxiii. (1895) pp. 268-72.

† Pogg. Ann., cxxxii. p. 361.

simple grating. The angle a of divergence of the maxima of the second order is given by the equation

$$\sin a = \frac{\lambda}{e},$$

 $\lambda$  being the wave-length of the light, and *e* the linear separation of the elements of the structure considered.

In order to show the rôle of the wave-length in these phenomena of diffraction, the author examines a valve of *Pleurosigma angulatum* with a dry objective with aperture a = 0.90, while projecting successively on the mirror of the Microscope the different parts of a spectrum.

If u denote the 1/2 angle of aperture of the objective, in order that the Microscope may give us an image of a structure composed of elements of which the linear separation is e, we must have for the axial illumination

$$\sin u = \frac{\lambda}{e},$$

and since the numerical aperture  $a = n \sin u$  where n is the index of refraction of the medium between the objective and the object,

$$a=n\sin u=\frac{\lambda}{e}.$$

For illumination at its maximum of obliquity

$$a=n\,\sin u\,=\frac{\lambda}{2\,e},$$

whence

$$e=rac{\lambda}{2a},$$

which is the formula of Helmholtz and Abbe. The resolving power thus depends not on the magnification, but only on the wave-length and the aperture of the objective.

The extreme limits of wave-length which we can utilise in the actual conditions are  $\lambda = 0.40 \ \mu$  for direct observation and  $\lambda = 0.35 \ \mu$  for photography. The angle of aperture of the objective cannot exceed 140°-160°, and the highest numerical aperture is 1.6. In these conditions the Microscope can show us elements of structure distant  $0.17 \ \mu$  from each other, i. e. 5800 elements to the millimetre, with white light. With violet light  $\lambda = 0.44 \ \mu$  of the spectrum we reach to  $e = 0.14 \ \mu$ , with 7000 elements to the millimetre; with photography  $\lambda = 0.35 \ \mu$ , e = 0.10, i. e. 10,000 elements to the millimetre.

For the resolution of molecules, if we take for the dimension of the molecule 1/1000,000 mm., i.e.  $e = 0.001 \ \mu$ , we should require apertures equal to 175 to 220 or wave-lengths of  $0.005 \ \mu$ . This shows clearly that the vision or photography of molecules by means of the Microscope is a thing physically impossible.

#### (6) Miscellaneous.

Microchemical Analysis.\*-The publication of this volume of Prof. Behrens marks a decided advance in a method of qualitative analysis which bids fair to rival and in many cases to supersede the old blowpipe processes. To the mineralogist and petrologist, who is often compelled to work on very limited amounts of material, these microchemical methods of analysis have become almost indispensable.

The two best known previous works on the subject, by Haushofer and Klément and Renard, in which the attempt is first made to give distinctive microchemical reactions for each element, leave much to be desired in the case of the distinction between many closely related elements. In the present case it can hardly be said that these difficulties have received their complete solution, but, as the author implies, the subject is still in its infancy, and the main object of the book is to give a short review of the rudiments of microchemical analysis and of the progress which has been made within the last ten years, and at the same time to point out the problems which need solution in the further development of this new branch of chemistry.

The book is divided into two parts. In the first, after a short nistorical introduction, the author states the general principles which have guided him in the choice of the microchemical reactions which are given for each element later on. Then follows a description of the apparatus and a list of the reagents used. With regard to the Microscope, any instrument which allows of magnifications from 50 to 200 times, and is provided with Nicol prisms and arrangements for measuring angles of crystals, and extinction angles, and for the observation of the optic axes in convergent polarised light, can be used. The author particularly recommends for microchemical work the Microscope (7b) of Seibert.

The greater portion of the first part of the book (100 pp.) is occupied with the description of the different microchemical reactions for each The second part treats of the systematic use of these reactions element. for the investigation of mixed compounds, such as minerals, rocks (in section and in powder), alloys, &c.

Manual of Microscopy.<sup>†</sup>-This book is what it professes to be, a guide to scientific microscopy, provided that we are content to know only of the instruments, workers, and methods of Germany. Although aiming at meeting the wants of schools of medicine and pharmacy, and giving all the information required in practical microscopical work by the student of biology, whether in botany or zoology, the student is given no information that would lead him to suppose that any other nation beside Germany had ever devised or employed a Microscope, or added anything to our knowledge of microscopic biology.

The book is admirably arranged and carefully written; it is accurate, and in some points exhaustive; but to the practical microscopist who has followed the history of optical theory and practice during the last

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<sup>\* &#</sup>x27;Anleitung zur Mikrochemischen Analyse,' von H. Behrens, Hamburg and Leipzig, 1895, 8vo, 224 pp., 92 figs. † Das Mikroskop. Ein Leifaden der wissenschaftlichen Mikroskopie, von Dr.

A. Zimmermann, Leipzig and Wien, 8vo, 1895, 334 pp., 231 figs.

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two decades, and their influence upon the development of lenses, and the subsequent construction of the Microscope, it leaves very much to be desired. It is true that it may be fairly looked upon as a handbook; but for this very reason, if only in the interests of the student, allusions might with some show of reason be expected to what has been done in other countries and by other workers than the always industrious Germans.

The students contemplated by the author of this book are certainly those who may be supposed to use it thoroughly, and from whom, in many cases at least, may be expected not only a repetition of class work, but the prosecution of discovery and research. It may be quite right to explain what the immersion system of lenses is as concisely as is compatible with practical usefulness to the user of the book; this has been done; but both water immersion and homogeneous immersion have a history of uncommon importance, and especially the latter imparts a distinct educational value to even its modes of employment. Certainly we may not charge the error of ignoring other workers than those of Germany on the practical deviser of the homogeneous system, for he has very frankly admitted the influence of one English worker in giving it origin.

Again, in the discussion of the influence of the cover-glass as a means of introducing aberration, and the method of correcting it, we doubt whether the student is benefited by the omission of its history, any more than in similar circumstances an English, American, or French student would be benefited by a description of the apochromatic system of lenses, without reference to the laborious discovery of the Abbe-Schott glass which has done so much for the modern Microscope.

This is even more apparent in the consideration of the value and application of that most powerful instrument in modern microscopy, the condenser—the only means by which the high qualities of modern lenses can be efficiently made manifest.

Those who have followed the history of the microscopy of the last twenty years know that even under the enlightened guidance of Abbe, although objectives and eye-pieces have been gradually brought to a higher and higher quality, and their corrections have been made more complete, yet in German centres the value, nay, indispensable importance of the substage condenser was not perceived.

It may be frankly admitted that the broad scientific value of the Microscope was understood earlier, and more largely from the first, on the Continent than in England; but also from the very earliest times its highest possibilities have been most fully seen and brought out in this country. It is so to-day. The very highest critical images of the most delicate objects have been demonstrated by English, and mostly by amateur microscopy. But this has always been due to the persistent use of the substage condenser, an instrument which has been steadily progressing in optical qualities to meet the improvement in objectglasses and general optical systems.

Without going back to the time of Tulley, it is enough to start in 1850 with the "Gillett condenser," so generally and long used by English microscopists; and from then until now the leading opticians have applied their very best powers to perfect the condenser as the one method by which the higher qualities of lenses could be brought out, until we were recently provided with an apochromatic form of this instrument by the firm of Powell and Lealand.

But it was not until 1873 that even Abbe saw the need of the provision of a condenser; and this arose from the fact that critical images became a necessity by the inauguration of the science of bacteriology. But the condenser as he thus for the first time made it was simply a chromatic one: by no means of value where the objectives were of the highest order and corrected on the best modern principles. Hence it became slowly manifest even in Germany, where the condenser had for so long been decmed only a toy, that an achromatic condenser must be constructed, and we are indebted to Abbe for the introduction, in 1888, of a practical form of this. But there had existed in England for many years several forms equally simple, and decidedly more suited to English stands.

But with what slowness the necessity for bringing out the higher possibilities of the finest recent objectives comes to the German maker may be seen by the fact that the firm of Zeiss have, as is so well known, constructed an objective beautifully corrected, on the apochromatic principle, having a N.A. of 1.63. And for the illumination of this they provide a chromatic condenser—a condenser wholly without corrections —having an aperture equal to the object-glass; so that unless we use an absolutely achromatic source of light, we introduce the most vicious aberrations. But in the treatise before us the substage condenser is taken for granted. It has no history; it goes with the German Microscope, which the student is directed to use, as naturally as he is to use a knife with a microtome.

There is much on the theory and practice of illuminating that is of value, and the mechanical stage, for so many years looked upon as one of the unnecessary luxuries of the English Microscope, is carefully described, as made by Reichert, who follows Zeiss in the practical adoption of the English model.

The micro-camera apparatus of Winkel, Thoma, and Edinger are introduced with profit, as we know that such apparatus for drawing is of great service to the student; and the section on the use of the polariscope is specially clear and practical.

There is a chapter on Photomicrography; and the remainder (109 pp. out of 321) is occupied with a description of the apparatus for and methods of preparing, cutting and mounting, for study and preservation, the material and objects employed for demonstration and research, which covers in the usual way the requirements of the student.

Lens Work for Amateurs.\*—A workshop receipt-book by a workmap who evidently understands nothing of the theory of optical instruments. In one part of this work there is a pretence of much theoretical knowledge, but a comparison with the English translation of 'Naegeli and Schwendener on the Microscope' reveals that both the text and figures have been abstracted wholesale.

When the ipsissima verba of Naegeli and Schwendener's text are left for purposes of condensation, the blunders made by the author show

\* H. Orford, 'Lens Work for Amateurs,' London, 1894 (1895). Svo, xv. and 231 pp., illust.

that he has quite failed to understand the meaning of the passages he has been so liberally quoting.

Where the author adheres strictly to his own subject, viz. the grinding, polishing, and mounting of lenses, the work will be found full of tips, wrinkles, dodges, and all that kind of information which is so useful to an amateur.

Dental Microscopy.\*-This work, which the author states is the first written on the subject, is not intended to teach dental histology, but simply the methods of research as at present in vogue in this special branch of minute anatomy, and which are only cursorily treated in the majority of ordinary text-books. There is a preliminary chapter on the Microscope and its accessories, so sketchy and imperfect, however, that it had been better omitted altogether. One or two extracts will serve to justify this opinion. Mr. Smith advises the purchase of a substage with focusing and swinging (sic) adjustments and an Abbe condenser, but all the subsequent reference made to the latter is that it should be used with the 1/6 objective in conjunction with the concave mirror and a small diaphragm. It is little wonder, therefore, that the author is of opinion, "It will be some time before the beginner can manipulate the substage condenser in a satisfactory manner," and thinks "it is unnecessary to add a long account of it; experience in its use, as throughout microscopy, is by far the best instructor." In recommending the student to use low powers only for many weeks, Mr. Smith remarks that with these "the tendency towards errors of refraction of light is considerably lessened," whatever this may mean, and that with high powers "there is greater fear of spherical and chromatic aberration." This is indeed Microscopy as she is taught. When, however, we come to the essential and larger part of Mr. Smith's work, viz. the preparation of hard and soft dental tissues, there is little to find fault with and much to commend; the methods advised are those found most suitable by the latest investigators here and abroad, and nothing of consequence appears to have been omitted in the processes of decalcifying, hardening, and staining. The book is illustrated by eight lithograph plates, and there is the inevitable chapter on photomicrography. It will, no doubt, be of service to such as are specially concerned with the minute structure of the teeth.

Lacquering of Microscope Tubes and Stands.<sup>†</sup>—Some practice is required in order to successfully lacquer a Microscope tube. The best process is to close the ends with cork and immerse in hot water. Before applying new lacquer the old must be removed by means of a scap solution containing benzol and alcohol (1 part of olive oil scap, 3 parts of 94 to 95 per cent. alcohol, 3–4 parts benzol, 1 part of caustic potash). The tube is then thoroughly cleaned and polished and placed in the hot water. The lacquer, which consists of red shellac dissolved in 95 per cent. alcohol in the proportion of 1 : 10, is poured into a deep saucer. The tube is then taken out of the water, carefully dried, and the lacquer applied with a broad brush.

\* A. Hopewell Smith, L.R.C.P., M.R.C.S., &c., 'Dental Microscopy,' London, 8vo, 1895.

† Central-Ztg. f. Optik u. Mechanik, xvi. (1895) pp. 55-6

The following solutions for different colours have given good results :---

Light golden yellow:—1 part shellac, 1 part turmeric, 4 parts alcohol.

Golden yellow :-25 parts shellac, 4 parts turmeric, 1 part dragon'sblood, 70 parts 95 per cent. alcohol.

Dark golden red :-2 parts orleans, 30 parts turmeric powder, 3 parts red sandalwood, 480 parts 95 per cent. spirit, allowed to stand with frequent stirring for 2-3 days; 60 parts shellac, 15 parts sandarac, 15 parts mastic, 15 parts Canada balsam are then added, and, after all is dissolved, 10 parts of oil of turpentine.

American Microscopical Society.-A circular has been sent us announcing the next meeting of this Society. It will be held at Cornell University, in Ithaca, N.Y., on August 21st, 22nd, and 23rd. It is pointed out that the equipment of the University in all lines for carrying on microscopic work should add to the attractiveness of Ithaca as a place of meeting. As nearly all the opticians have expressed a desire to be present and make an exhibit of their Microscopes and microscopical apparatus, the members will have an opportunity to see all the new and standard apparatus. Following what they call "our prototype," the Royal Microscopical Society of London, the American Society not only pub-lishes papers upon the Microscope, its manipulations and accessories, but also on the results of investigations in which the Microscope plays an important part. It is added that the University possesses one of Rogers' dividing engines, and the Department of Physics has kindly promised to show the members exactly how microtomes are made. A special feature of the coming meeting will be the setting apart of one or more sessions for the reading of papers and methods and the demonstration of special or new methods.

#### $\beta$ . Technique.\*

#### (1) Collecting Objects, including Culture Processes.

Cultivation Media containing Thyroid Extract,<sup>†</sup>—Dr. K. Kopp finds that nutrient media containing some thyroid gland juice possess an important value for discriminating between certain micro-organisms. The media are prepared from fresh sheep's thyroid, which is carefully freed from fat, and having been finely minced is soaked in an equal bulk of sterile water for about three hours. The infusion is, after straining through linen, filtered through a porcelain filter. The extract thus obtained is mixed with an equal bulk of 20 per cent. gelatin which contains 1 per cent. of salt. One per cent.<sup>\*</sup>agar is made on similar lines, 2 grm. agar, 6 grm. glycerin, 1 grm. salt, and 100 grm. water are mixed with an equal volume of the extract, both being at a temperature of 40° C.

Cultivation media containing thyroid extract were found to exert

<sup>\*</sup> This subdivision contains (1) Collecting Objects, including Culture Processes; (2) Preparing Objects; (3) Cutting, including Imbedding and Microtomes; (4) Staining and Injecting; (5) Mounting, including slides, preservative fluids, &c.; (6) Miscellaneous.

<sup>†</sup> Centralbl. f. Bakteriol. u. Parasitenk., 1º Abt., xvii. (1895) pp. 81-3.

on some micro-organisms an inhibitory effect, on others the growth appearances were characteristic, in other cases the addition appeared to have little or no influence. The author gives a table showing the results obtained by cultivating *Bacterium coli commune* and *Bacillus* typhosus in these media.

Cultivation Media containing Egg-Yolk.\*—Dr. Nastiukoff prepares three kinds of nutrient media in the following way:—

(1) Egg-yolk solution: 1 litre of distilled water plus 0.5 ccm. 10 per cent. solution of caustic soda plus 100 ccm. of egg-yolk. The yolk is freed from any albumen by Bunge's method (filtration through blottingpaper). The 10 per cent. yolk solution is placed in a flask and steamed for about 2 hours, and then, having been allowed to stand for a day, is filtered. The filtrate is distributed into test-tubes and sterilised in the usual way. The fluid is clear, and by transmitted light yellow; by reflected, greenish.

(2) To 300 ccm. of yolk are added 100 of 1 per cent. caustic soda solution and 600 distilled water, the ingredients (the latter two having been previously sterilised) being stirred the while with a glass rod. The 30 per cent. yolk solution may be at once distributed into test-tubes and heated in a water-bath to  $75^{\circ}$ -85°. Even at  $75^{\circ}$  the solution solidifies without impairment of its transparency, and by repeatedly heating to  $85^{\circ}$  it is sufficiently sterilised.

(3) To 1 litre of the 10 per cent. yolk solution are added—15-20 ccm. agar or 80-100 gelatin, and the whole boiled until it is completely dissolved. The thick opaque mass is filtered through a hot-water filter. Or the ordinary neutral agar or gelatin in equal parts may be mixed with the egg-yolk solution, the mixture evaporated down to one-half by boiling and afterwards filtered. Before filtering it is better that the fluid stand for about 2 hours in the steamer, in which the steri-Fig. 66. Isation is afterwards performed. Thus prepared the medium resembles very much the ordinary agar or gelatin.

According to the author, these media are excellent for influenza bacilli, gonococci, diphtheria bacilli, glanders, typhoid, cholera, and other pathogenic micro-organisms.

Simple Method for cultivating Anaerobic Organisms in Liquid Media.<sup>†</sup>—Dr. Ad. Schmidt finds that a simple test-tube, into which a bent glass tube fits through the intermediation of a caoutchouc plug, is an excellent apparatus for making anaerobic cultures in liquid media. The test-tube should be made of thick glass and hold about 30 ccm. The tube should be about 15 cm. long, and its long arm fits into the plug, the lower end being a few mm. above the bottom of the plug. After the test-tube is filled with some medium, the plug is put in and the fluid rises into the glass tube (see fig. 66). The apparatus is then sterilised, and should, owing to evaporation, the media sink down, more of the fluid must be put in. The apparatus is inoculated by

carefully removing the stopper. The author says the apparatus works very well.

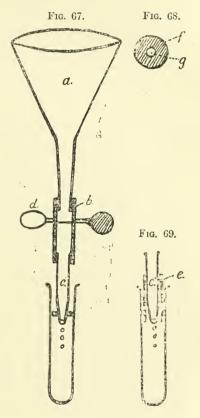
\* Wratsch, 1893, No. 3, pp. 33 and 4. See Centralbl. f. Bakteriol. u. Parasitenk., 1° Abt., xvii. (1895) pp. 492-3.

Centralbl. f. Bakteriol. u. Parasitenk., 1e Abt., xvii. (1895) pp. 460-1 (1 fig.).

Funnel for filling Test-tubes with Media.<sup>\*</sup>—Dr. J. J. van Hest uses the following apparatus for distributing cultivation media in testtubes, &c. :—Fig. 67 shows an enamelled or glass funnel, attached to the pipe of which is a caoutchouc tube b, which can be closed by a pinchcock. To the tube is joined an outflow glass tube c for dipping into the

test-tube. When the medium is poured in through the funnel its flow is stopped at any desired point by the pinch-cock. Smearing of the tube and consequent damage to the apparatus for cultivation often happens, but this may be avoided by the simple device shown in fig. 69, which is nothing more than a second tube placed between c and the test-tube. Over the end of c is pushed a caoutchouc ring (fig. 67) at a distance of 4-6 mm. from the end. The apparatus shown in fig. 68 can be used without any special practice, but that in fig. 67 requires some acquaintance, at least for manipulating quickly.

Modified Papin's Digester.†-Dr. J. J. Van Hest has used for some years a steriliser which can reach 100°-130°. The apparatus consists of a cylindrical iron kettle with an iron top. The steam is produced from heating a small quantity of water. The lid is fixed on by means of a number of small screw clamps, and between the pan and its cover is a thin layer of rubber for the purpose of effectually closing the apparatus. In the middle of the lid is a valve which can be loaded with leaden discs.



The pieces to be sterilised are placed on a sieve or stand at the bottom of the apparatus. The top is then screwed on and the valve kept open until the thermometer marks 100°. By this time all the air has been driven out, and the valve is then closed by placing on it as many lead discs as are necessary to attain the desired temperature. When this is reached, the excess of steam escapes through the valve. The temperature is pretty constant.

# (2) Preparing Objects.

Intercellular Bridges.<sup>‡</sup>—Herr H. Boheman fixed smooth musclecells with Heidenhain's corrosive sublimate, Kultschitzky's solution of

- \* Centralbl. f. Bakteriol. u. Parasitenk., 1º Abt., xvii. (1895) pp. 462-3 (3 figs.).
- † Tom. cit., pp. 463-4 (1 fig.). ‡ Anat. Anzeig., x. (1894) pp. 305-15 (6 figs.).

bichromate of potash and copper sulphate in 50 per cent. alcohol, Rabl's platinum chloride and picric acid; and succeeded well in demonstrating the intercellular bridges. After fixing he stained his preparations with a solution of rubin (patent-acid rubin .25 grm., 2 per cent. acetic acid, saturated picric acid 100 grm.), using 3-4 ccm. to 100 ccm. of absolute alcohol. Golgi's silver method also gave good results.

Examination of Central Nervous System of Desmognathus fusca.\* -Mr. P. A. Fish decalcifies the cranium of this salamander by Perenyi's mixture or Gage's decalcifier. The latter is made up by adding 3 ccm. of strong nitric acid to 100 ccm. 70 per cent. alcohol, and gives uniformly good results. The tissue was fixed either by potassium bichromate, corrosive sublimate, picric alcohol, or a mixture which may be called picroaceto sublimate. This last is composed as follows :- 50 per cent. alcohol, 1000 ccm.; glacial acetic acid, 5 ccm.; corrosive sublimate, 5 grm.; picric acid, 1 grm. This gave most satisfactory results, and brought out many histological details that were not demonstrable by the other methods. For staining, Delafield's hæmatoxylin gave most excellent results. Contra-staining with Van Gieson's picro-fuchsin gave most brilliant effects. Herrick's modification, which consists in adding a tablet of .5 of a gram of corrosive sublimate and .5 grm. of ammonium chloride to about half a litre of the stain, was found to work very satisfactorily. Another satisfactory modification is the addition of 1 ccm. of glacial acetic acid and 1 ccm. of a saturated aqueous solution of corrosive sublimate to 100 ccm. of the hæmatoxylin. For histological details the short silver nitrate method of Golgi and Weigert's hæmatoxylin method were mainly employed. In the silver method the author used 3 per cent. potassium bichromate 100 ccm., 1 per cent. osmic acid 20 ccm. The tissue was allowed to remain in this mixture from 24 to 48 hours, according to the temperature. It was then rinsed rapidly, and placed in a weak solution of 1/4 per cent. silver nitrate for 15 or 20 minutes. This was changed a couple of times until the fluid remained clear. It was then immersed in a 3/4 per cent. silver nitrate solution, and left there for two days or longer. The object was cleared and cut in a mixture of 3 parts red oil of thyme and 1 part of castor oil.

If the specimen is stained in toto the method is a very expeditious It was found that the sections, after absorbing the superfluous oil one. with tissue paper, could be fixed to the slide by means of a drop or two of ether-alcohol, and that they might then be passed through the various alcohols, and stained similarly to the paraffin or ordinary collodion methods. Any tendency toward crumbling or tearing on the part of the sections may be obviated by painting the cut surface of the object with a thin layer of 1 per cent. collodion before making each cut; this will also enable one to cut much thinner sections.

Preparation of Fish Eggs.<sup>†</sup>-Mr. J. T. Cunningham has found it very difficult to preserve fish ova satisfactorily for cutting, as the germinal vesicle, or the protoplasm, or both, are more or less destroyed by the preserving reagents. He finds that the much-vaunted mixtures of Flemming, namely, chromic, osmic, and acetic acids are not satisfactory,

<sup>\*</sup> Journ. Morphol., x. (1895) pp. 234–5.
† Journ. Mar. Biol. Ass., iii. (1895) p. 270.

and that the fault lies in the acetic acid, which causes shrinkage of the nucleus and destruction of its delicate reticulum. Chromic and osmic acids alone, when the chromic is not too strong, have a good effect, but at the same time have the disadvantages of contracting the nucleoli, preventing staining, and making the yolk hard and brittle.

Examination of Nephridia of Phylactolæmatous Polyzoa. \*— Mr. A. Oka, in his investigation, first stupefied the colonies with a fluid prepared after Cori's receipt (10 per cent. solution of methyl-alcohol in 0.75 per cent. salt solution with a few drops of chloroform), and then fixed them with Flemming's fluid. After washing in rain-water they were gradually hardened in alcohol. For staining he used Boehmer's hamatoxylin, sometimes in connection with alcoholic solution of eosin. The stupefying fluid used seems to work especially well upon such forms as these.

Examination of Segmenting Ova of Isopods.<sup>†</sup>—Prof. J. P. McMurrich recommends, for developing eggs of Isopods, fixing in alcoholic picro-sulphuric acid, staining in Kleinenberg's hæmatoxylin, and washing in acid alcohol until all the stain is removed from the yolk. The egg should then be cleared in oil of cloves, and examined as a transparent object.

Study of Young American Sponges.<sup>‡</sup>—Mr. H. V. Wilson found that the young *Esperella* is, after metamorphosis, so thin that it cannot be scraped off the dish without injury. He therefore coated the dishes with a thin layer of paraffin; collodion was also used. For fixing purposes, he found that very much the best fluid was the mixture of acetic acid, alcohol, and osmic acid recommended by Zacharias. He allowed this to act for from 10 to 20 minutes. Kleinenberg's picric method was of use for special points, as it often preserved the individual cells in a more natural and uncontracted condition than the Zacharias fluid, but in general it disassociated the elements too much.

A new Method of Entrapping, Killing, Imbedding, and Orienting Infusoria and other small objects for the Microtome.§—Mr. J. A. Ryder, feeling the want of a reliable method for this purpose, has, after trying a number of devices, hit upon a plan that is not only very simple, but is also capable of wide application, since organisms as small as  $12.5 \mu$  in diameter may be cut and held. He makes a filter from thin slices of elder-pith, which are perforated at pretty regular intervals by openings caused by cutting through the very thin cellulose walls of certain of the pith-cells. A good supply of the little pith-filters should be cut, and kept in stock in a pill-box ready for use at any time. The next step is to take some ordinary good white filtering-paper cut in discs or squares about 1 in. in diameter.

A small drop of water on the end of a wooden toothpick should be used to moisten a point at the centre of one of these paper discs or squares, so as to make a damp area just about the area of one of the discs of pith. A wire 1/16 in. thick should be heated at one end, and with it some melted paraffin should be placed on the paper disc. This

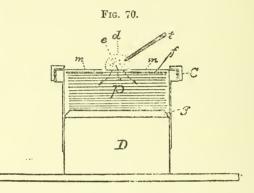
<sup>\*</sup> Zool. Mag., 1895, pp. 25. † Zool. Anzeig., xviii. (1895) pp. 109-11.

t Journ. Morphol., ix. (1894) pp. 286 and 7.

<sup>§</sup> Amer. Natural., xxix. (1895) pp. 194-8 (1 fig.).

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may be heated with the hot wire so as to saturate the whole of the paper disc, except the central moistened spot, which must be left unsaturated, with paraffin. The next step is to prepare a discoidal pad about 1 in. in diameter composed of ten to twenty superposed thicknesses of filterpaper; upon this a disc of filter-paper is superposed. Fig. 70 shows the arrangement of the parts. The cap-ring C of a large-sized live-box holds a perforated mica cover mm in place. The perforation of the mica should be a little larger than the disc of paper e, immediately below which lies a disc of filter-paper f, which is saturated with paraffin, except at its centre. Then follows the pad of several thicknesses of filter-paper P.

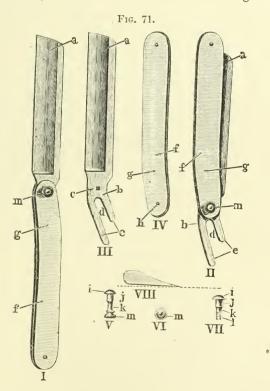


The mode of preparation is as follows:—Place P upon the glass disc qof the live-box or compressor, then lay f upon P, then put the cap C in place and slip it close down over the drum D, so as to hold f firmly down upon P. Next moisten the central exposed part of f, that is the part not saturated with paraffin, with a little water, pick up one of the little discs of filter-pith by one edge with a fine forceps, and lay it down on the moist centre of f, when it will at once flatten out and adhere to f, and just neatly cover the central area not saturated with paraffin. On the apparatus so prepared place a drop of water D swarming with animalcules from a vigorous culture on e, when it will be found that the water will be drawn rapidly through e and f into P, in the direction of the arrows. In this way several drops of water may have a large part of their population separated out and caught upon the surface of e. To kill the contents of D it is only necessary to add a little saturated corrosive solution or 1 per cent. osmic acid. The animalcules are at once precipitated by the killing agents upon the upper surface of e, where they are caught and held in the meshes formed by the pith-cells. The filter e should now be gently removed by means of a needle and forceps. With gentle handling Mr. Ryder finds that Ciliates will remain attached to e, and may be passed through a dozen reagents without becoming detached, and that the pith-disc may be imbedded in paraffin very readily by the watch-glass method. The pith-discs may also be mounted entire, and in this way most instructive preparations may be prepared. Staining is also entirely under control, and with this method it has been found possible to cut 18 longitudinal serial sections and 50 transverse serial sections of Paramæcium with a thickness of 2.5 to 5  $\mu$ . The fixation of the sections on the slide may be effected by Gustav Mann's albumen method. Mr. Ryder adds that a very simple form of this apparatus for holding the filter-paper in position may be obtained from Messrs. Charles Lentz and Sons, of Philadelphia.

#### (3) Cutting, including Imbedding and Microtomes.

Sectioning Fern-Prothallia.<sup>\*</sup>—Mr. M. B. Thomas describes a process for imbedding fern-prothallia and other delicate objects in collodion, and sectioning with the microtome. Before imbedding the object must be well hardened and dehydrated. It is then placed in a solution of 1.5 grm. gun-cotton in 100 ccm. of equal parts of ether and alcohol; the liquid is slowly evaporated until solid collodion remains behind. The subsequent treatment is much the same as that of objects imbedded in paraffin.

Universal Razor for Microscopists.<sup>†</sup>—Dr. Éternod has devised, for the use of students, a razor which can be used for cutting sections either



the blade with the handle. The blade (fig. 71, I a, II a, III a) is of English steel of mean temper. The cutting part is perfectly straight and of as simple a form as possible. The blade is plane and slightly oblique on its lower surface, but a little hollow on the upper surface (VIII). Its back is thick and rounded on two sides for convenience of sharpening. The form of the back also allows of the blade being easily fastened by means of the clamp to the different sliding microtomes. The shank of the blade (II b, III b) is perforated by a square hole (III c), and is terminated by a fork set obliquely to the rest of the blade. The slot (II d, III d) between the two branches of the foot (II e, III e) serves to receive the pressure-screw of certain sliding microtomes (e. g. Schanze's).

The handle (If, IIf, IVf), of aluminium, has a slight bend (Ig, IIg, IVg), so as to diminish the quantity of metal of the handle and to preserve the blade better when it is closed. The head of the handle is pierced on one side with a square hole, and on the other with a round one (IVh), in which the bolt fits. The steel bolt (V, VI, VII;Im, IIm) consists of (1) the head at its lower end (Vi, VIIii), (2) a square part (Vj, VIIj), (3) a cylindrical part (Vk, VIIh), (4) a screw part (VIIl), and lastly (5) a milled head (Im, IIm, Vm, VIm). The square part of the bolt j fits into the square hole on one side of the handle and into the square hole of the blade (IIIc), while the cylindrical part fits into the round hole on the other side of the handle.

Rapid Method for Hardening and Sectioning.\*—Dr. J. Coats gives a method for hardening, of which the principles are rapid hardening in alcohol, cutting with the microtome without removing the alcohol and without freezing the tissue, and rapid staining. Put shortly, his method is as follows:—(1) Select an illustrative part of the fresh tissue and remove a slice with a sharp knife. (2) Place in absolute alcohol and heat the vessel in a water-bath to about 40° C. for half an hour to an hour. (3) Dry the tissue and place it on the freezing plate of the microtome in a large drop of anise oil. (4) Freeze the anise oil, which freezes at a high temperature, and cut sections. The upper surface of the knife may be moistened with alcohol while cutting. (5) Place in alcohol to remove anise oil. (6) Float out in water and place on slide for staining. (7) Stain by any approved rapid method, and mount.

## (4) Staining and Injecting.

Examination of Wandering Cells of the Frog.<sup>†</sup>—Dr. A. A. Kanthack and Mr. W. B. Hardy give an account of the methods employed by them in the investigation of the wandering cells of the Frog. They find that differences in the texture of cell-substance are brought into marked prominence by the use of iodine, and they say that this reagent cannot be too highly praised in this connection. The nuclear type of the various cells has been studied with the aid of a solution of methyl-green slightly acidulated with acetic acid, and with a drop of osmic acid added. The nuclear characters were also shown by treatment with an alkaline alcohol-osmic acid solution of methylen-blue. With this solution

\* Amer. Natural., xxviii. (1894) pp. 827-9.

† Phil. Trans., clxxxv. B. (1894) pp. 283 and 4.

eosinophile granules are entirely uncoloured and unchanged. Amphophile granules are stained blue, while the basophile granules appear violet with daylight, and brilliant rose with yellow light. The substance which produces the rose-coloured modification of methylen-blue does so whether it be present as granules in the cell-substance, or dissolved in the surrounding fluid. The study of the living cells and their behaviour towards noxious or innocuous substances has been carried out by injecting various substances into the lymph-spaces of the frog, and withdrawing drops of lymph for examination at varying intervals of time, and by hanging drops. The hanging drops were suspended on the under side of a cover-slip in moist chambers, sufficiently large to provide air enough for the needs of a small drop of lymph for about ten hours, without introducing a fresh supply. The cover-slips used were always carefully cleaned with acid and absolute alcohol, and then sterilised by heat, immediately before use.

Gold Impregnation.\*-Mr. C. L. Bristol gives an account of a method of using formic acid and gold chloride suggested to him by Miss J. B. Platt. He has used it in tracing the nervous system of Nephelis lateralis, and has found it reliable. In leech tissues it differentiates all nerve tissue, though the results with other tissues are poor. It has been used successfully on larval vertebrate material also, by varying the strength of the formic acid, or the time of its application. The following process was employed :- The leech is put into 20 or 30 times its bulk of 10 per cent. formic acid, and left from 3 to 5 minutes. It dies well extended. It must now be transferred without washing to 1 per cent. gold chloride for 25 minutes, then put without washing into 1 per cent. formic acid for 24 hours, or until reduction is complete. This is indicated by a rich purple colour over the whole specimen. It must now be washed slightly in tap water; run up through the alcohols to chloroform, saturated with hard paraffin. When the impregnation appears to be very slight, stain the sections on the slide with erythrosin or some other deep red anilin stain for contrast. These sections will often show the most exquisite details. Transparent larvæ 5 to 10 mm. long require a milder treatment, such as 5 per cent. formic acid for 2 or 3 minutes, 1 or  $\frac{1}{2}$  per cent. gold chloride for 10 minutes, weak formic acid for 1 to 4 hours. The reduction of the gold chloride may be The gold chloride stopped at any point by transferring to alcohol. solution was exposed to sunlight for some time before using; this may not be an essential factor in the process, but it has been suggested that failure to ripen the solution by sunning may be the cause of many of the failures in gold staining.

Brain of Pike.<sup>†</sup>—Herr L. Neumayer used the chrom-osmic-silver method as modified by Ramón y Cajal. The objects were placed in a mixture of 1 part of 1 per cent. osmic acid and 4 parts of 2 per cent. potassium bichromate solution. He left the objects two days in the osmic acid and potassium bichromate solution, two days in 0.75 nitrate solution. To add a few drops of formic acid to the silver nitrate solution is very advantageous. A second or third repetition of the whole

\* Amer. Natural., xxviii. (1894) pp. 825-6.

† Arch. f. Mikr. Anat., xliv. (1895) pp. 345-65 (1 pl.).

process for one day in each solution was attended with good results. Thereafter the brain was placed for an hour in absolute alcohol, and cut in celloidin.

Minute Structure of Electric Organ in Torpedo.\*-Herr N. Iwanzoff tried over a score of different fixatives, &c., but his best results were obtained when he used chromic salts-especially bichromate of potashafter injection with osmic acid, and stained with aqueous solution of hæmatoxylin. Osmic acid and Flemming's fluid alone, or these with subsequent use of potassium-gold-chloride, were employed when staining was not desired. His paper includes a long discussion of various methods used by Fritsch and others.

Differentiation of Hypodermic Glands.<sup>†</sup>-M. E. G. Racovitza describes a means of differentiating hypodermic glands in Annelids (Micronereis variegata), when one wishes to study the order of appearance, number, or distribution, without investigating minute structure.

The Annelid is placed gently in water on a slide, flooded with acetic acid, washed in distilled water for a few seconds, and then placed in mixture A, viz. :- Solution of methyl-green (Grubler) 0.3 per cent., 1 vol.; fluid of Ripart and Petit, 1 vol. The latter consists of chloride of copper 0.3 grm., acetate of copper 0.3 grm., crystallised acetic acid 1 grm., camphorated water, not saturated, 75 grm., distilled water 75 grm. The Annelid is coloured uniformly green in a few minutes; but after 3-6 days the body generally is decolorised, while the glands are intense blue.

Then the specimens are placed in mixture B, viz. :--Pure glycerin, 1 vol.; fluid of Ripart and Petit, 1 vol. Thereafter the body is clear as crystal, except the glands, which are intensely blue.

## (5) Mounting, including Slides, Preservative Fluids, &c.

Formol as a Preserving Fluid. ‡-Mr. F. C. Kenyon has an interesting essay on this subject. The first half of it is a free translation of a paper by Prof. T. Blum.§ It has been found that the properties of formol as a preservative medium may be summed up as follows :--Animal objects are hardened without shrinking, and without losing their microscopic structure or staining properties; the natural form and colour is preserved; the eyes remain much clearer than in alcohol; the mucus of slime-producing animals is not coagulated and remains transparent; the colouring matter of blood in tissues apparently disappears. but may be quickly restored by a high per cent. alcohol; plant structures are more or less well preserved; most fruits keep well; chlorophyll is not extracted, but after a long action of the fluid delicate leaves may be changed; other colouring matters are retained for varying periods with individual plants; microscopic sections of plants that have been a long time in formol give fine preparations. Dilute formol is incombustible, and is much cheaper than alcohol. To the results of Blum the author adds some experiments of other German naturalists and some notes of his own. He obtained some startling effects with salamander blood.

\* Bull. Soc. Imp. Nat. Moscou, 1895, pp. 407-89 (3 pls.).
† Arch. Zool. Expér., ii. (1894) No. 3, pp. viii-x.
‡ Amer. Natural., xxix. (1895) pp. 82-91.
§ Ber. Senck. Naturf. Gesell. Frankf. a. M., 1894, pp. 195.

A few drops of blood were placed on a slide in a 1 per cent. solution of formaldehyde and watched under the Microscope. The corpuscles, and especially the nuclei, were seen to swell rapidly; the nuclei became as large almost as the original corpuscles, and were seen to pop out of the corpuscle like a grape from its skin. The envelopes then became very pale and finally disappeared from view, but the nuclei remained very Staining with Ehrlich-Biondi mixture showed that the body distinct. of the corpuscles had simply been rendered very transparent by the solution, while immersion in alcohol coagulated the fibrin into an opaque straw-yellow mass, and brought the corpuscle faintly back into view. This explains the phenomenon of the return of the colour of bloodvessels noted by Blum, and is due to the coagulation of the fibrin, which may also be stained somewhat by the colour drawn from the corpuscles. Mr. Kenyon concludes that it may be said, for general purposes, that solutions of at least more than 2 per cent. must be used in order to avoid the swelling and the decolorisation of specimens; solutions of from 4 to 8 per cent. will give the best results. For histological purposes, formaldehyde combined with alcohol will give better results than either Weak (1 to 2 per cent.) solutions, by swelling nuclei, may used alone. serve the very important special purpose of demonstrating the presence of cells not otherwise readily distinguished.

Preservation of Marine Animals.\*-Mr. W. A. Redenbaugh has applied the method of preservation suggested by T. Tullberg a few years ago, in which a novel use was made of magnesium sulphate. A method which was found successful with various marine animals was the following :- The animal is killed by slowly adding a ·1 per cent. solution of chromic acid until the water contains from .03 to .05 per cent. of the acid. Sections of tentacles showed that the cells were not attacked by the substances employed. The author has found that the desired results may be obtained in a much simpler manner than that described by Tullberg. Complete stupefaction of the organism must be produced, so that when it is removed to a killing fluid no contraction takes place. The process, however, must not be carried on so slowly that maceration may ensue. For Cœlenterates the most beautiful results were obtained with sea anemones, which ordinarily are so difficult to preserve in a well-expanded condition. They were allowed to expand in a dish with as little water as possible; crystals of magnesium sulphate were then placed in the bottom of the dish and allowed to dissolve slowly until a saturated solution was obtained. A large *Physalia* treated in this way was preserved in 4 per cent. formalin with all the tentacles and polyps fully extended. For Echinoderms the method was found to be equally successful. Annelids, placed in a saturated solution of Epsom salts, became in a very short time perfectly limp, and were easily extended upon a glass plate, and treated with a fixing reagent. Balanoglossus, when taken soon after being collected, was thus preserved in nearly a perfect state. Good results were also obtained with Gephyreans and Nemerteans. For Ascidians it is best to add the saturated solution of sulphate intermittently with a pipette. A number of experiments were made upon Ctenophores, and successful results were obtained with the

\* Amer. Natural., xxix. (1895) pp. 399-401.

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following method :- To a solution of equal parts of 2 per cent. formalin and Perenvi's fluid was added enough common salt to increase the density of the mixture to that of sea-water, that is, until a Ctenophore placed in it barely floated. This adjustment of the density of the surrounding medium prevented the Ctenophores collapsing of their own weight. After remaining for about half an hour in this fluid they were transferred to 4 per cent. formalin. The density of each had been increased by the addition either of Epsom salts or common salt. The former is probably better than the latter for increasing the density of the fluid.

Preserving Brains.\*-Dr. A. Lanzillotti-Buonsanti advocates the use of formalin for preserving brains. This fluid contains 40 per cent. of formic aldehyde, and may be mixed with water in various proportions. The author left dogs' brains for 10-12 days in a 2 per cent. solution, and then placed them for a like period in glycerin. The results were better than with Giacomini's method.

Wiese's Preserving Fluid.<sup>†</sup>—Dr. A. B. Meyer publishes a letter from Wiese to the effect that his fluid is disappointing only because collectors use it carelessly and not enough. Each object must have 3-4 times its own volume of preserving fluid. But the letter does not make clear whether even a large quantity of the fluid will prevent soft specimens, such as fishes, from falling to pieces, which was what Meyer complained of.

## (6) Miscellaneous.

Standard Unit of Size for Micro-organisms. 1-Mr. G. C. Whipple, after pointing out that the results of the microscopical examinations of water by the ordinary methods are often misleading owing to the custom of recording the number of organisms present in a cubic centimetre of water examined, without regard to their character or size, shows that it is advisable to use some unit by which the actual quantity of animal and plant matter present may be expressed; such a standard would be a square 20 microns wide, having an area of 400 square microns. This is suggested because it has already been used in estimating amorphous matter, because it is about the size of several common organisms, and because it is a unit whose size can be easily carried in the mind.

It will be found of great advantage to have the ocular micrometer divided as follows: the square, which should cover one square millimetre on the stage of the Microscope, is first divided into four equal squares, and each of these quarters subdivided into smaller squares, each of which is equivalent to twenty-five standard units.

Two plates are given showing how there is a general parallelism between the profile (curve) of the albuminoid ammonia and that of the organisms expressed in terms of the standard unit, while the curve of the actual numbers of the organisms does not accord well with the chemical analyses.

It should be noted that this volumetric method has been used for over two years at the Boston Waterworks, and several other biological laboratories have since adopted it.

- \* Mon. Zool. Ital., v. (1894) pp. 273-5.
- <sup>+</sup> Zool, Anzeig., xviii. (1895) pp. 122-5.
   <sup>+</sup> Amer. Mon. Micr. Journ., xv. (1894) pp. 377-81 (2 pls.).

Microscopic Characters of Powdered Drugs.\*-In discussing the botany of the present edition of the British 'Pharmacopœia,' in view of the approaching revision of that work, Dr. J. Reynolds Green says, "In the present volume no description is given of the microscopic characters of powdered drugs. When we consider that in the present day most pharmacists purchase many drugs in the condition of powders, it appears desirable that they should be able to identify the powder apart from the drug in the unaltered condition. This can be done only by microscopic examination. Many of the constituents of vegetable tissues have a very definite value in this respect; many starches are extremely distinctive; the tissues of seeds are very unlike those of roots, and so on. It is only necessary here to call attention to a few illustrations of this point. Ipecacuanha, when powdered, can be recognised with tolerable certainty by two points-the vascular elements are in the form of perforated tracheids, true vessels being absent; the starch grains are of a peculiar appearance. Jalap in powder again shows distinctive starch grains, peculiar crystals, and numerous resin-containing cells. The powdered seeds of strychnos nux vomica would be easily identified by the application of the micro-chemical tests for brucine and strychnine.

Many powders need special investigation, of course, to enable accurate diagnostic tests to be ascertained; for example, rhubarb, liquorice, and gentian powders. A new 'Pharmacopœia' would be much improved by the introduction of the distinctive features which such powders present, and these could without much difficulty be determined. Within the last ten years much more accurate information has been obtained on the point of the definite micro-chemical tests which various constituents of vegetable tissues respond to. Those which are given in the present · Pharmacopœia' are often negative, and some depend on very variable constituents. In the case of many decoctions it is stated that they do or do not give an indigo-blue colour with iodine. This test is of little value as given; it, of course, is only a test for the presence of starch in the drug under discussion. The presence of starch in many roots will depend on their condition when gathered; the quantity of starch, and therefore the depth of the colour of the decoction on addition of the iodine, will vary very greatly from time to time. In many cases where this test was said to give a negative result, the reason for applying it is not apparent. The value of the test will depend on its distinguishing the drug under examination from another, for which it might be mistaken, which does contain starch. In such a case, why should not the two drugs be mentioned as differing in this respect?

Some of the micro-chemical tests given are not only not distinctive, but actually inaccurate. 'Thus Cusparia bark is said not to give an arterial red coloration when the fractured surface is touched with nitric acid. In many cases this treatment does produce a red colour, though perhaps not quite that of arterial blood. Presumably this test is meant to distinguish Cusparia bark from that of nux vomica.''

Intrahydraulic Pressure as a new Method of Investigation.<sup>†</sup>— Dr. Stanislaus von Stein has constructed an apparatus with which to

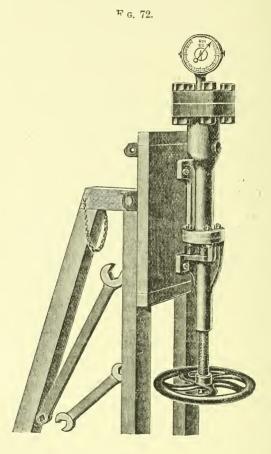
\* Brit. Med. Journ., No. 1786 (1895) pp. 668-70.

+ Zeitschr. f. wiss. Mikr., xi. (1894) pp. 321-6.

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systematically study the effect of pressure on certain tissues and organs. A strong wooden frame which is fastened to the floor by screws, carries a board which can move about a horizontal axis, so that it can be adjusted either vertically (as in the figure) or horizontally. To this board is screwed the hydraulic press made of phosphor-bronze, at the lower end of which is seen the wheel with the pressure screw (310 mm. long). Above this is the piston, the press-cylinder with thick walls, and the strong



cover with four screws (these three parts are together 355 mm. long) surmounted by the manometer which is adjusted up to 2000 atmospheres. The total weight of the whole apparatus amounts to 60 kg.

To use the instrument, the four screws of the cover are loosened with the long spanner seen in the figure, the bolts removed and the cover with the manometer taken off. The space (38 mm. in diameter and 179 mm. long) is then filled with water up to the brim. The cover is replaced with the cylindrical projecting piece (11 mm. high and 56 mm. broad) on its lower end fitting into the opening. Between the edges and the projecting piece which is provided with a row of concentric grooves, is a copper ring which is pressed into the grooves when the cover is screwed on. With good packing of the piston the apparatus keeps a pressure of 700 atmospheres for a considerable time. With phosphorbronze a pressure of 1500 atmospheres could not be obtained, the cylinders breaking at 1100 to 1200 atmospheres. For these high pressures a steel cylinder must be used. The author describes several interesting experiments which can be made with the apparatus to show to what high pressure living things and cell-protoplasm may be subjected without being destroyed.

Scientific Models.\*—Prof. W. His pleads for the higher appreciation of scientific models, which he thinks as worthy of citation as printed documents. Thus Hochstetter should have referred in a recent criticism to His's models of eye-development prepared by Ziegler. For there, His has in visible clearness expressed his conclusions as to the complex relations of the different parts.

\* Anat. Anzeig., x. (1895) pp. 358-60.

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The late Prof. Williamson.—We regret to have to report the death of one of our Honorary Fellows, Dr. W. C. Williamson, Emeritus Professor of Botany in Owens College, Manchester. Prof. Williamson, who was born in 1816, was best known for his extensive and long-continued researches on the minute structure of fossil plants. His capacities as a naturalist were so well known that, on the foundation of Owens College in 1851, he was made Professor of Natural History, and was required to teach every branch of biology. As the college increased, special chairs for various of these branches were founded, so that at last he was able to confine himself to botany. In 1892 he resigned his chair, and, settling near London, was an occasional attendant at our meetings.

The following is a list of Prof. Williamson's contributions to the Transactions of this Society :---

On the Structure of the Shell and Soft Animal of Polystomella crispa; with some Remarks on the Zoological Position of the Foraminifera. (Trans. Micr. Soc. London, ii. (1849) pp. 159-80, pls. 25 and 26.)

On the Minute Structure of the Calcareous Shells of some Recent Species of Foraminifera. (Trans. Micr. Soc. London, iii. (1852) pp. 105 -28, pls. 17 and 18.)

Further Elucidations of the Structure of Volvox Globator. (Trans. Micr. Soc. London, N.S. i. (1853) pp. 45-56, pl. VI.)

On the Minute Structure of a Species of Faujasira. (Trans. Micr. Soc. London, N.S. i. (1853) pp. 87–92, pl. X.)

On the Structure and Affinities of some Exogenous Stems from the Coal-measures. (Monthly Micr. Journal, ii. (1869) pp. 66-72, pl. XX.)

The late Dr. Anthony.—Our late Fellow, who died on June 1st last, at Edgbaston, Birmingham, served for several years on the Council of the Society, and was deeply interested in its welfare. He was M.D. of the University of Cambridge, some time Fellow of Caius College, and F.R.C.P. Dr. Anthony was eighty-one years of age at the time of his death.

### B. Technique.\*

#### (1) Collecting Objects, including Culture Processes.

Resistance of Lower Vertebrates to Artificial Microbic Infection.<sup>†</sup>—In his experiments on the mode of resistance of certain lower Vertebrates to artificial microbic invasion M. A. Mesnil used *Gobio fluviatilis*, *Perca fluviatilis*, *Carassius auratus*, and the frog. The microbes were the bacilli of anthrax and of mouse septicaemia. The general conclusions at which the author arrived were that Teleostean fishes resist the bacillus of anthrax by means of phagocytosis. The lymph of these fish has neither bactericidal nor attenuating properties. The eosinophilous, or rather, cells with granulations, are either absent or insignificant in number. The frog, in which eosinophilous cells are frequently abundant, resists anthrax in the same manner as fish. By means of its phagocytes it also incorporates the living virulent microbes of mouse

\* This subdivision contains (1) Collecting Objects, including Culture Processes; (2) Preparing Objects; (3) Cutting, including Imbedding and Microtomes;
(4) Staining and Injecting; (5) Mounting, including slides, preservative fluids, &c.
(6) Miscellaneous.
† Ann. Inst. Pasteur, ix. (1895) pp. 301-47.

septicæmia. At  $35^{\circ}$  the frog is still immune to anthrax, the bacteria being destroyed as at  $20^{\circ}$ . But in frogs which die less than 2–3 days after inoculation, in consequence of the leucocytes being paralysed, the microbes which are still alive develop freely in the blood and organs. When microbes are introduced directly into the blood they are seized on much more quickly than those injected into the dorsal sac, and in this destruction the macrophages of the liver play an important part. The eosinophilous cells of the frog and lizard are endowed with a positive chemiotaxis, less than that of ordinary phagocytes; the granulation cells of the lizard are capable of commencing the digestion of the bacteria which they have incorporated.

Procuring and transmitting Diphtheritic Discharges for Examination.\*—Prof. D. J. Hamilton advises an ordinary 3/4 in. wide testtube, fitted with a tight plug of cotton into which is inserted the handle of a small goat's hair brush. The brush must be firmly fixed, and its end reach nearly to the bottom of the tube. The whole is, after having been sterilised, ready for use. In starting a cultivation on blood serum or other medium the brush is merely rubbed over the surface.

Preparing Kidney-Juice Cultivation Media.<sup>†</sup>—Dr. O. Henssen prepares kidney-juice media in the following way. The kidneys must be taken out directly the animal has been killed, and after the capsule has been removed the organs are finely minced and then pulped on a mortar. An equal bulk of water is then poured over the mass, and after three hours the juice is squeezed out through a fine linen cloth. The juice is then drawn through a sterilised clay filter by means of a water pump. The filtrate is of a straw yellow colour. It is warmed to 40° C., and then mixed with an equal bulk of  $2\frac{1}{2}$  per cent. agar, heated to the same temperature. The mixture is then allowed to stand for 24 hours, in order to see if it be sterile, after which it may be used for cultivating micro-organisms.

Apparatus for clearing Agar without Filtration.<sup>‡</sup>—Dr. M. Bleisch has devised an apparatus for obtaining clear agar without filtering. The apparatus consists of a cylindrical glass vessel holding about two litres, and furnished at top and bottom with a tube, the lower one being closed with a perforated caoutchouc plug, and kept firm by means of a clamp. The perforation is for the passage of a glass tube of suitable length, capable of being pushed up and down. The lower end is supported by a brass plate, fixing it to the apparatus, which in its turn is supported on a stand. Before using it the tube is pushed up as high as it will go, and the lower aperture closed by tightening the clamp. Warm water should then be poured into the apparatus to see if all the parts fit tight, and after the water has been removed agar is poured in through a funnel until the top of the glass rod just remains above the level of the fluid. The apparatus is then placed in an incubator at 50°-60° C. After the sediment has settled (and the deposition may be hastened if phosphate be used instead of carbonate of sodium), the apparatus is removed and placed at a convenient height. To the lower end of the

† Centralbl. f. Bakteriol. u. Parasitenk., 1te Abt., xvii. (1895) p. 403.

‡ Tom. cit., pp. 360-2.

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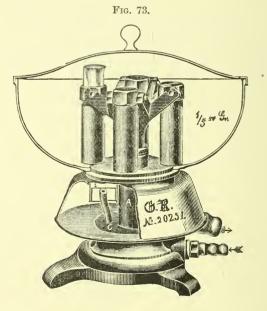
<sup>\*</sup> Brit. Med. Journ., 1895, i. p. 298.

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glass tube is fitted on a rubber tube with clamp. The tube is then pulled down a few centimetres. The clamp on the tube is then opened, and the agar runs through into some suitable recipient. The glass tube must be lowered from time to time as the agar descends until the whole of the clear fluid is removed. On account of the relative waste of material it is not advisable to make the apparatus smaller.

Simple Method for isolating Bacteria on Agar and Blood-Serum.\* —Prof. G. Banti fills the ordinary test-tubes and tubes 2–3 cm. wide with the requisite quantity of agar, and allows it to set obliquely, the condensation water running to the bottom. The material to be examined is then diluted with a few ccm. of bouillon or sterile water, and 1, 2, or 3 drops of the mixture placed in the condensation water of three tubes or more, according to the dilution of the material, or the number of bacteria present, which of course must previously be determined by microscopical examination. In some cases, of course, it is not necessary to dilute the material, but it can be placed straight away in the condensation water. When the two ingredients are mixed by shaking gently the condensation water is allowed to flow over the agar surface, and then the tube placed upright in the incubator. This simple procedure is said to possess all the advantages of plates without their inconvenience.

Centrifuging Agar.<sup>†</sup>—Dr. C. S. Haegler uses a centrifuge for preparing agar; by this procedure the clarifying is shorter and simpler



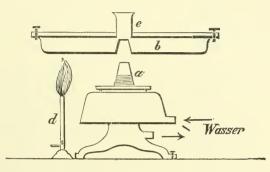
than clearing gelatin. The centrifuge is driven by water-power, and to the stem is screwed on a plate (fig.  $74 \ b$ ), having a lid fastened on

\* Centralbl. f. Bakteriol. u. Parasitenk., 1te Abt., xvii. (1895) pp. 556-7.

† Tom. cit., pp. 558-60 (2 figs.).

with screws, a rubber band interposing; while the agar mixture is being prepared in a steamer, autoclave, or over the open fire, the plate is gently warmed with a Bunsen's burner, and when ready the agar mixture is poured on to the plate (or pan b) through the funnel opening e. The centrifuge is set in motion, and the burner removed. In about half an hour the agar is cooled and set, and on examining the contents of the pan it will be found that the agar is perfectly clear, all the particles

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having been sedimented in a layer of 2-3 mm. at the periphery. It only remains to cut off the sedimented layer to obtain a mass of perfectly clear agar, which is then distributed into test-tubes, &c.

There are two points of importance in the manipulation: the first is that the pan should be thoroughly warmed before the agar is poured in; and the second, that when this is done, the centrifuge should be made to work up as fast as possible, otherwise the agar may be slightly opalescent. The centrifuge used by the author is Runne's (fig. 73).

Preparation of Mallein.<sup>\*</sup>—Herren Fr. Hutyra and H. Preisz prepare mallein in the following way. After the virulence of the glanders bacilli has been considerably increased by repeated transferences through guinea-pigs, cultures are made on potatoes in capsules. When the cultures and the potatoes have become quite dry and black they are placed in a glass vessel and just covered with a fluid composed of equal parts of distilled water and glycerin and 3–5 per cent. chloride of mercury. After having been incubated for 10–14 days at  $37^{\circ} \cdot 5$ , the mixture is filtered and then steam sterilised for an hour. The fluid obtained is dark brown, but there is no connection between darkness of colour and intensity of action. For one dose 0.3-0.5 ccm. sufficed, and this the authors diluted with 0.5 per cent. carbolic acid up to 3.0 ccm.

Bacteriological Examination of Water.† — Herr G. Marpmann points out that for practical purposes the bacteria in water may be divided into two great classes, pathogenic and sewage bacteria. The pathogenic include the typhoid bacilli, pyogenic cocci, and the cholera vibrio;

<sup>\*</sup> Centralbl. f. Bakteriol. u. Parasitenk., 1te Abt., xvii. (1895) p. 341.

<sup>†</sup> Tom. cit., pp. 362-7.

the sewage, the coli group, and numerous peptonising putrefaction bacteria. By cultivation these may be split up into other groups: (i.) the typhoid which will grow on agar containing 0.2 per cent. citric acid, but not on agar containing 2 per cent. carbonate of soda; (ii.) the cholera vibrios which will grow on agar containing 2 per cent. carbonate of soda; and (iii.) the sewage bacteria which will not grow on agar with 0.2 per cent. citric acid.

The agar cultures are kept at 30°, and acid or alkaline meat broth may also be used at the same temperature. If there should be a small number of germs their number should be increased by mixing equal volumes of the water and sterilised bouillon and keeping it for 24 hours at 30°. Samples should then be tested in acid and alkaline media. If there be any clouding, then (i.) growth on alkaline agar at 30°-37° C. = cadaver bacteria; (ii.) growth on acid gelatin at 20°-22° C. = typhoid bacteria. Though the same results may be arrived at by examining the bouillon yet the recognition is safer and easier on gelatin or agar surface.

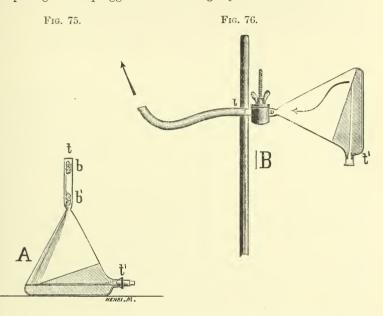
The next step is to test by plate cultivations; this will determine the species, e.g. typhoid, coli bacteria, vibrios, pyogenic organisms, and possibly anthrax. Should a colony be suspected to be typhoid, inoculate on saccharated medium, then there will be development of gas with coli organisms but not with typhoid. If there be growth on citric acid media, then it is typhoid. If there be growth at room temperature on alkaline gelatin then sewage bacteria are present. If at room temperature an alkaline cultivation shows no growth, but does on incubation, the water is impure from excrement and cadaver bacteria are present. Any water which on alkaline gelatin or agar gives colonies is harmful.

Simple Appliance for Bacteriological Examination of Air.\*-Dr. P. Miquel describes a very ingenious device for examining air. The apparatus consists of a conical flask A (fig. 75), furnished with vertical and lateral tubes t, t'. The vertical tube is slightly constricted, and in it are placed two cotton-wool wads, b, b', the latter lying against the constricted neck, and being intended to catch any germs which may have escaped through the medium. In the lateral tube t' is fitted a thin glass rod ("pointe de verre") kept in position in t' by means of a cork or caoutchouc plug. The diameter of the pointed glass rod used for flasks of 7-8 cm. at the base, varies from 1 to 2 mm. The flasks are prepared for use by first inserting the cotton-wool plugs, and then pouring in as much gelatin as will suffice, when the flask is slightly inclined, to cover the rod, except at the end furthest from t'. The vessels are then sterilised for half an hour at 110°. When the apparatus is removed from the autoclave, the flasks are tilted so that the tip of the glass rod just projects above the level of the gelatin. In this position it is left until the gelatin is set.

When the apparatus is used, it is clamped to a support (fig. 76, B) the tube t' being directed downwards. To the tube t is fixed a caoutchoue tube in connection with an aspirator. The glass rod is now withdrawn, and this is effected by first gently warming the plug which supports it in the tube t', and carefully pulling it out, giving it at the same time a

\* Ann. de Microgr., vii. (1895) pp. 103-9 (2 figs.).

turn to the right and to the left. Thus a long narrow passage is left in the medium. The aspirator is now set going at the rate of 1 litre every 2 or 3 minutes, and when a sufficient volume of air has passed the opening at t' is plugged with cork slightly burnt in the flame.



When the flasks reach the laboratory, the gelatin is liquefied at  $35^{\circ}$ -37°. Then the flasks are shaken so as to disseminate the germs throughout the medium. The gelatin is allowed to set again, and after an incubation of 30 days the colonies are counted.

**Preserving Streptococci Cultures.**\*—Dr. J. Petruschky records the fact that he has kept two cultures of Streptococcus for six months, without any loss of virulence, and this was done by merely keeping the tubes in an ice safe.

ABEL, R., & A. DRÄER-Das Hühnerei als Kulturmedium für Choleravibrionen. (Fowl's Eggs as Culture Media for Cholera-vibrios.)

Zeitschr. f. Hygiene, XIX. (1895) pp. 61-74.

- AUSSET, E.—De l'influence de la température dans l'analyse bactériologique des eaux. (The influence of Temperature in the Bacteriological Examination of Water.) Compt. Rend. Soc. Biol., 1895, No. 3, pp. 58-9.
- BALL, M. V.—A new Culture Medium for the Bacillus of Diphtheria and other Bacteria. Med. News, 1894, p. 581.
- BONHOFF—Untersuchungen über Giftbildung verschiedener Vibrionen in Hühnereiern. (Investigations into the formation of Poisons by various Vibrios in Hen's Eggs.) Arch. f. Hygiene, XXII. (1895) p. 351.

FROTHINGHAM, L.---Laboratory Guide for the Bacteriologist. Philadelphia, 1895, 8vo, 61 pp.

\* Centralbl. f. Bakteriol. u. Parasitenk., 1te Abt., xvii. (1895) pp. 551-2.

KIEFER-Zur Kultur des Gonococcus Neisser. (Cultivation of G. neisseri.) Berl. Klin. Wochenschr., 1895, pp. 332-4.

NOVY, F. G .- Directions for Laboratory Works in Bacteriology, for the use of the Medical Class in the University of Michigan. Ann Arbor, 1894, 8vo, 209 pp.

VAN ERMENGEM, E., & E. SUGG-Recherches sur la valeur de la formaline à titre de désinfectant. (The Value of Formalin as Disinfectant.) Arch. de Pharmacodynamie, I. fasc. 2, 3.

WHIPPLE, G. C.-A Standard Unit of Size for Micro-organisms.

Amer. Mon. Micr. Journ., 1894, pp. 377-81.

#### (2) Preparing Objects.

New Method of Studying Cell Motion.\*-Dr. C. Lennard describes a method of studying cell motion which consists in the making of a consecutive series of instantaneous photomicrographs of the same microscopic field taken at definite intervals, and the comparative study The author's first series exhibits the amceboid motion of of the series. the white blood-corpuscles, and the change in shape and motion with relation to the surrounding stationary and identical folds is well marked. The second series shows the path of the white blood-corpuscle in forcing its way through a mass of red adherent blood-corpuscles. The third series is said to be of marked interest, a white has seized on a red corpuscle, and a series of photomicrographs shows that it has dragged it through a considerable distance in a field which is proved to be stationary and identical in all the photomicrographs. The fourth series shows motion in a red blood-corpuscle; the fifth and sixth show the passage of the red blood-corpuscle from a capillary in which blood is in motion, and from one in which the blood is at rest. The seventh series shows a capillary; along the inner surface of the wall of this there may be seen white corpuscles in which the series indicates movement.

Experiments on Frogs' Eggs.<sup>†</sup>-Mr. T. H. Morgan, in his experiments on the blastomeres of the Frog's egg, followed in general the directions given by Roux, but some eggs were turned after the operation so that the white pole was upwards. As the membrane was pierced, the egg could easily be turned into any desired position, and it would, in most cases, hold this position until subsequent cleavages had taken place. The eggs after the operation were placed on a piece of moistened glass and kept for ten hours under a bell-jar in a saturated atmosphere. If, immediately after the operation, the eggs be simply thrown into a dish of water, they assume all possible positions with respect to the vertical, and tend to hold that position through subsequent stages of development.

Study of Necturus maculatus.<sup>‡</sup>-Mr. B. F. Kingsbury, in his examination of the histological structure of the intestine of this Amphibian, found that the best way to harden the tissues was to use mercuric chloride, picric alcohol, or Ehrlich's fluid. 'The first of these was made by adding a saturated solution of mercuric chloride to normal salt The tissue was hardened in this for 1-12 hours and then solution.

\* Proc. Acad. Nat. Sci. Philad., 1895, pp. 38 and 9.

† Anat. Anzeig., x. (1895) pp. 623 and 4.
 ‡ Proc. Amer. Mier. Soc., xvi. (1894) pp. 43-5.

thoroughly washed for 12–24 hours in 67 per cent. alcohol to which a little gum camphor was added to hasten the removal of the mercuric chloride; so long as tincture of iodine is decolorised when added to the alcohol, the washing should be continued; unless the chloride is entirely washed out, crystals will form in the specimen. For the outlines and relations of cells Ehrlich's fluid gave good results, but for details of structure was generally found to be inferior.

The castor-thyme method introduced by Fish was employed in sectioning. The object was imbedded in collodion. The hardened tissue was dehydrated for 12-24 hours in 95 per cent. alcohol, placed in 2 per cent. collodion for 1 day, 3 per cent. collodion for 1 day, and 5 per cent. collodion for 1 day. The collodion was then hardened in chloroform for 1-3 hours and cleared in a mixture of castor oil and three parts of red oil of thyme. When transferred to the slide the superfluous oil was absorbed and the collodion melted with equal parts of alcohol and ether. The Ehrlich-Biondi stain gave excellent results, especially in a study of glands and leucocytes, because of its high selective power. The best results with a nuclear stain were found by the use of an aqueous solution of hæmatoxylin.

Amitosis in Ovaries of Hemiptera.\*—Herr F. Preusse fixed in concentrated cold sublimate (5–10 minutes) or chrom-osmio-acetic acid, after Flemming and Carnoy, or Kleinenberg's picro-sulphuric; and stained most successfully with hæmatoxylin.

Histology of Trematodes.<sup>+</sup>—Dr. A. Schuberg, in his investigation into the histology of Trematoda, made use of the method of methylenblue staining, and prepared sections from carefully preserved material. In his staining experiments he washed out the flukes from a still warm liver by means of a fine brush, and conveyed them at once into a methylen-blue solution (1/3-1/4 per cent. methylen-blue plus 0.75 per cent. salt solution), which was warmed to about the temperature of the body, the solution being kept warm; the objects were left in it for 4-5 hours or longer. The best results, i.e. a staining of as many of the nervous elements as possible, were obtained with animals which were just beginning to die. For control observations he made use of sections. For the investigation of nervous elements he recommends the osmic acid-acetic method of v. Mährenthal. The fresh flukes were killed in a 1 per cent. solution of osmic acid, which must be contained under a cover-glass supported by a wax foot, so that the animals may be kept well flattened out. After the removal of the cover-glass they were left for 6-12 hours in the osmic acid, so that it should completely penetrate them. They were then brought directly into the acetic acid, washed with water, and imbedded in paraffin. As all the tissues blacken completely, the sections must not be too thick.

Investigation of Gyrodactylus.<sup>‡</sup>—To fix these parasites Dr. L. Kathariner made use of cold sublimate solution or of a solution as hot as  $50^{\circ}$ . This was succeeded by chrom-acetic acid and osmic acid, while borax-carmine and hæmatoxylin were the staining reagents. Control

‡ Tom. cit., p. 128.

<sup>\*</sup> Zeitschr. f. wiss. Zool., lix. (1895) pp. 305-49 (2 pls.).

<sup>+</sup> Arbeit. Zool. Zoot. Inst. Würzburg, x. (1895) pp. 168-70.

observations were in many cases effected by the investigation of the living animal.

Division of Ceratium.\*—Herr R. Lauterborn preserved his specimens in Flemming's mixture, picrosulphuric acid, 45 per cent. iodinealcohol, &c. But the first was best. In it the specimens were left for 10 minutes and then washed. Treatment with gradations of alcohol followed. For staining, Delafield's hæmatoxylin gave clearest results for the chromatin of the nucleus. Paraffin sections were also cut for the study of the centrosomes, Heidenhain's method of staining being followed.

Study of Sporozoa.—Mr. J. Jackson Clarke<sup>†</sup> confirms the statement of Wolters, that Flemming's fluid does not give good results with Gregarines. He adopted a method which he found most satisfactory, not only for Gregarines but for Coccidium oviforme, and for animal tissues in general. Small portions of the tissue are placed for 24 hours in Foà's reagent, which is a mixture of equal parts of a saturated solution of corrosive sublimate in normal saline solution, and a 5 per cent. solution of bichromate of potassium or Müller's fluid. 'The material is then transferred for a day to running water and afterwards placed on successive days in 30, 60, and 90 per cent. alcohol. After this they are placed in absolute, and after saturation with chloroform are imbedded in paraffin. Care must be taken that the bath does not reach a temperature higher than 50°. The sections were cut with a Minot's microtome, and fixed on the slide with albumen and glycerin. They were stained with Ehrlich's acid hæmatoxylin diluted with distilled water, and when they had assumed a brownish-pink colour were transferred to a bath of tepid tap water and left for at least two hours. Then for two or three minutes they were stained with a solution of Grübler's water-soluble eosin, dehydrated, cleared by xylol and mounted in the usual way. The last mentioned solution was obtained by dropping a few drops of a strong alcoholic solution into a watch-glass filled with distilled water.

Mr. G. Eisen,<sup>‡</sup> in his investigations of the life history of Spermatobium, found that the following method was superior to any other. The hosts were stained *in toto* in very weak Delafield's hæmatoxylin, or in Ehrlich's ammonia hæmatoxylin. After hardening and sectioning in paraffin the slide fixing consisted simply of distilled water or of formalin and gelatin (1/2 per cent.) in water. This fixing is used as follows :—(1) Cover the space of the entire glass on the slide with several drops of the fixing so that the sections will float; warm gently over a plate until the paraffin becomes slightly transparent, but not so long that it begins to melt; let the fixing harden in the air during at least four hours, or better during the night; the paraffin should be dissolved in pure turpentine or xylol; when the latter is at last removed by alcohol the slides are stained by a saturated solution of orange G in 33 per cent. alcohol. Attention is called to the very great advantages of gum Thus in xylol as a mounting medium, for it gives images which are far superior to those found with Canada balsam or dammar.

<sup>\*</sup> Zeitschr. f. wiss. Zool., lix. (1895) pp. 167-90 (2 pls.).

<sup>+</sup> Quart. Journ. Micr. Sci., xxxvii. (1895) pp. 287-8.

<sup>‡</sup> Proc. Cal. Acad. Sci., v. (1895) pp. 4 and 5.

Demonstrating the Tubercle Bacillus in the Sputum.\*-M. J. Amann gives the result of a 12 years' experience of examining for tubercle bacilli in the sputum. All the sputum should be tested, and it is made homogeneous either by squeezing it between two ground-glass plates or by sedimentation. The former method requires no explanation ; with regard to sedimentation, the author points out that any chemical or physical means which are detrimental to the tinctorial properties of the bacilli should never be adopted, and his method for treating the mass is to place it in a glass vessel holding about 100 ccm. 2-4 volumes of distilled water are then poured over it so that the vessel is about half full. 1 ccm. of chloroform and some clean lead shot are then put in, and the vessel having been tightly plugged is shaken up for some minutes. The fluid is then placed in a conical wine-glass, or better still in a U-shaped glass tube, the bottom of which is narrowed to 2 mm., so that it resembles somewhat a Geissler's burette. 2 ccm. of phenol-fuchsin solution are then added and the apparatus shaken. The top is closed with a perforated caoutchouc plug, through the aperture of which is inserted a After standing for 24-48 hours the sediment will balloon syringe. have deposited, the supernatant fluid being quite clear. The bulb is then squeezed and the sediment flows out into a receptacle placed to receive it. The sediment can then be examined for epithelium or elastic fibres as well as for bacilli. The sedimentary sputum is next spread on slides, and when dry fixed either by heat or by immersion in equal volumes of ether and absolute alcohol, the latter process being preferred as it removes the fatty particles.

For staining the preparations (already partially coloured) use phenolfuchsin 1 grm., fuchsin 5 grm., 90 per cent. carbolic acid, and 95 ccm. hot distilled water. A few drops of this solution are poured on the slide, which is then heated till it vaporises over a spirit-lamp. The preparation is decolorised as follows:—It is first treated with 20 per cent. picro-sulphuric acid for 1/2-1 minute and then washed thoroughly in running water. It is next treated with the special solution, 15 grm. fluorescein, 15 grm. methylen-blue, 500 ccm. absolute alcohol, until the sputum is of a pale greenish-yellow colour. The next step is to stain the preparation with malachite-green in dilute aqueous solution, and this done it is dried at a temperature of  $60-80^\circ$ . While it is still warm cedar oil is dropped over one or two spots, and when cold the preparation is examined without the interposition of a cover-glass, though of course it can be mounted in the usual way.

### (3) Cutting, including Imbedding and Microtomes.

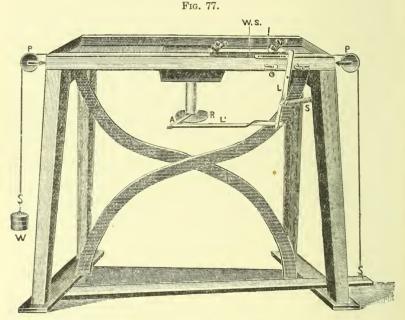
Microtome for Cutting Sections under Spirit.— Alexander Bruce, M.D., F.R.C.P.E., Lecturer on Pathology, Surgeons' Hall, Edinburgh, writes :— Most microscopists who have had occasion to use a sliding microtome to cut sections from tissues imbedded in celloidin have been met by several difficulties in their work. Among them may be mentioned :—(1) Friction of the cut section against the blade. This is obviated fairly well in the case of small sections by dropping spirit on the blade; but when the sections are of considerable size it is very

\* Centralbl. f. Bakteriol. u. Parasitenk., 1te Abt., xvii. (1895) pp. 513-22.

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difficult to bathe the knife with sufficient spirit to prevent them from tearing. (2) The vibration in the unsupported end of the blade. (3) The fact that, in most cases, a change in the obliquity of the edge of the blade, which may become necessary during cutting, is always accompanied by a change in its plane, and therefore by the loss of several sections. (4) The difficulty of adjusting the tissue to be cut to any desired plane.

I have devised an instrument to meet these difficulties so that it is possible—(1) to cut sections with a knife completely under spirit; (2) to support the knife by both ends; (3) to change the cutting edge of the knife to any obliquity desired without altering its plane; (4) to allow of ready adjustment of the tissue by means of a simple mechanism



Microtome seen from the side.

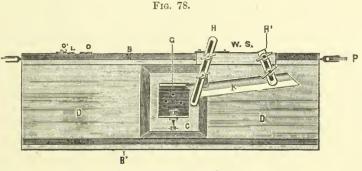
for holding the block of wood on which the tissue is mounted, and of its easy orientation by means of a ball-and-socket joint.

The instrument consists (figs. 77 and 78) essentially of the following parts:—(1) A long shallow trough (D D, fig. 78) for holding spirit. (2) A wedge slide W S, which moves along a groove (B, fig. 78) on one side of the trough, and supports the knife K by two horizontal arms H H. (3) A screw mechanism G passing through the centre of the trough for carrying and raising the tissue that is to be cut. (4) A treadle-arrangement (fig. 77) automatically raising the section.

The trough D is 36 in. long, 8 in. broad, and 1 in. deep. In the centre is a well C, 7 in. square and  $2\frac{1}{2}$  in. deep. To the middle of the floor of this well is attached the apparatus G for raising the tissue

to be cut. The object of the well is to give greater depth for the tissue than is possible with the shallow trough, and, at the same time, to economise spirit. The sides of the trough have two perfectly parallel wedge-shaped grooves for carrying the wedge-slides. The object of the double groove was to enable the knife, in cutting large sections, to be supported at both ends and from both sides of the trough; but since the instrument has been in use it has been found that the knife can be perfectly well supported from one wedge-slide alone. The ends of the trough are closed with a thin layer of oak.

The trough is made of cast iron in a single casting, and the grooves in the sides are planed by a planing machine. The wedge-slide W S is 10 inches long, and is made of cast-iron. The sliding surfaces have been grooved so as to diminish friction in working. To the upper surface two long lever-arms H H, made of gun-metal, can be fixed. Those are slotted throughout almost their entire length, so as to allow the knife to be placed at any desired degree of obliquity to the piece of tissue to be



Microtome seen from above.

cut. This adjustment can be effected with the greatest readiness, and the plane of the knife is thereby scarcely, if at all, altered.

The knife K is hollow ground. Its blade is 12 in. long and  $1\frac{3}{4}$  in. broad. Its ends have been cut obliquely so as to allow the point of the blade to come nearer the margin of the trough than would have been possible had the ends been rectangular. This is especially desirable when very large sections are to be cut. The knife can be fixed by a simple adjustable apparatus to the slotted arms (fig. 78).

The screw for raising the object has forty turns to the inch. To • its upper extremity is attached a ball-and-socket arrangement, into the former of which is screwed the clamp for holding the tissue. A special arrangement is provided for screwing the socket on to the ball to any desired degree of tightness. It is not difficult to screw the socket up so that it should be just so loose that the clamp can be easily moved into any desired position, and yet so tight that it shall retain that position during the process of cutting.

The screw is raised by means of a toothed wheel (R, fig. 77) at its lower end, and this wheel, again, is turned by means of an arrangement of levers thrown into action by the impact of the wedge-slide at the end of its back stroke (L L', fig. 77). This wheel has about 120 teeth in its circumference.

The wedge-slide is drawn backwards by a treadle T worked by the foot, acting through a cord which passes round a pulley P at one end of the trough. It is drawn forward by a cord and weight W attached to the opposite end of the wedge-slide and acting on a pulley P at the other end of the trough. The rate of movement of the slide is controlled partly by the amount of the weight attached to the cord, and partly by the foot hindering the rise of the treadle. The mechanism for raising the section is so arranged that the screw is rotated throughout the desired distance when the knife is at the end of its return journey and just in position to commence the cutting movement. When the wedge-slide is pulled backwards it strikes the upper end of a vertical lever arm L, which moves on a fulcrum fixed slightly below the side of the trough.

The other end of the lever moves forward a horizontal rod L, which is attached to an arm A moving round the centre of the toothed wheel. This arm bears a ratchet which carries the wheel round through the desired distance. This distance again is regulated by the travel of the lever-arm, the wheel being the further carried round the greater the movement of the lever, and vice versa. The movements of the arm can be easily and finely adjusted by two small slotted bars O O', attached by butterfly screws to the side of the trougb. When the knife moves away from the lever, its lower end, and with it the rod and ratchet, are drawn back by a spring attached to the leg of the instrument. The ratchet is thus ready to move round the wheel when next the wedgeslide strikes the lever. The general arrangement of the mechanism is seen in fig. 77.

The operator sits or stands at the side of the trough opposite to the groove in which the wedge-slide acts. He thus has both hands free for the manipulation of the sections.

The instrument is made by Mr. Alexander Frazer, 22 Teviot Place, Edinburgh. It is already in use in my laboratory, Surgeons' Hall, Edinburgh, and in the Universities of Edinburgh, Aberdeen, Dublin, and in Queen's College, Belfast.

Method for Marking Small Objects in Paraffin Imbedding.\*—It is often difficult, says Herr M. Samter, to discover the whereabouts of a small colourless object when imbedded in paraffin; but this may be surmounted by the following device. Paraffin is easily stained red by merely rubbing alkanin into it when melted. The object having been saturated with this red paraffin, is then imbedded in colourless paraffin through which its position is easily discernible.

Collodium as a Material for Imbedding.<sup>†</sup>—Prof. G. F. Atkinson describes in detail the best method of preparing the various stages in the life-history of Ferns, and of using collodium as the imbedding-material for sections made with the microtome.

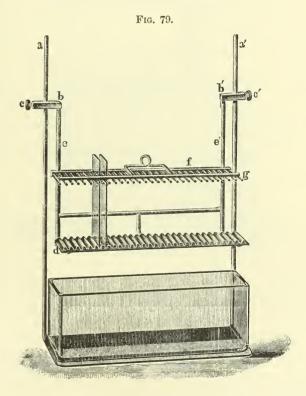
<sup>\*</sup> Zeitschr. f. wiss. Mikr., xi. (1895) pp. 469-71.

<sup>† &#</sup>x27;The Study of the Biology of Ferns, &c.,' New York, 1894, 134 pp. and 163 figs. See Bot. Centralbl., lxi. (1895) p. 95.

### (4) Staining and Injecting.

Slight Modification of Golgi's Method.\*—Prof. A. S. Dogiel has endeavoured to get over the well-known difficulty that blood-vessels and the ducts of glands are apt to be impregnated as well as the nerves in using Golgi's method. He injects at the outset with a saturated solution of Berlin-blue and gelatin, cools the subject in snow for 20-30 minutes, cuts off suitable portions, and treats with Golgi's bichromate of potash solution or with Ramon y Cajal's osmio-bichromic mixture. The ordinary procedure follows, or a red injection may be used, with subsequent treatment in the osmio-bichromic mixture. The injection-colours last for some time and obviate confusion.

Apparatus for Staining Serial Sections.<sup>†</sup>—Herr R. Borrmann has devised an apparatus for the convenient and rapid staining and further



manipulation of serial sections. It consists (figs. 79 and 80) of a base to support a glass rectangular vessel in which the various fluids are placed. From the base proceed two metal uprights a, a', to which are fixed by means of screws c c' the tray d, supported by means of the pieces

- \* Anat. Anzeig., x. (1895) pp. 555-7.
- † Zeitschr. f. wiss. Mikr., xi. (1895) pp. 459-64 (2 figs.).

 $b \ b'$  and  $e \ e'$ . The tray is grooved to prevent the slides touching at the bottom, while at the top is a toothed rackwork  $f \ g$ , much like a scullery plate rack. The method of using the apparatus is obvious from the illustration. The material of which the apparatus is made is brass, and

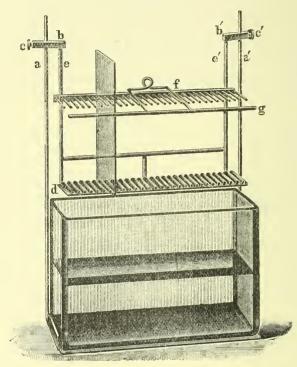


Fig. 80.

back to back it will hold sixty slides. The chief measurements are as follows :—Bottom 16 by 6 cm.; uprights, 25 cm.; tray, 15 by  $3 \cdot 5$  m. Of course the measurements vary with the size of the slides, as may be seen by comparing the two illustrations.

Staining the Plasma-sheath of Anthrax.\*—Herr F. Lüpke recommends a freshly prepared 0.2 per cent. solution of gentian-violet for staining anthrax, and makes this by taking a drop of a 10 per cent. alcoholic solution of gentian-violet and adding it to 50 drops of sterile water. The cover-glass preparation is then heated until the fluid begins to vaporise, after which it is washed with water.

Demonstrating the Nerve-fibres of the Vertebrate Heart. $\dagger$  — In studying the innervation of the vertebrate heart Drs. J. F. Hymans and

\* Deutsch. Tierärztl. Wochenschr., 1895, p. 23. See Centralbl. f. Bakteriol. u. Parasitenk., 1<sup>te</sup> Abt., xvii. (1895) p. 683.

† Arch. de Biol., xiii. (1895) pp. 619-70 (25 photo plates).

L. Demoor followed Golgi's method, though they took full advantage of the modifications suggested or introduced by other workers with the same procedure, such as Ramon y Cajal and Kallius. No details of the technique are given. They mention that the photographs, which are numerous and excellent, were taken on isochromatic plates with the oxyhydrogen light.

Demonstrating the Parasites of Artificial Carcinoma.\* - Prof. F. Sanfelice placed the pieces removed from the dog and fowl in alcohol, and when hard stained them in toto with lithium-carmine. The pieces were then treated with acid alcohol and absolute alcohol, imbedded in paraffin, and sectioned. The sections were stuck on with albumen and freed from paraffin with xylol, washed with absolute alcohol, and then placed in Ehrlich's fluid for 5-10 minutes. On removal they were bathed in distilled water, to which a few drops of 0.5 per cent. oxalic acid had been added. The excess of acid was next removed by washing with distilled water. The sections were dehydrated in absolute alcohol and mounted in xylol-balsam. By this method the parasites are stained violet and the tissues red. Another method by which good results were obtained was, after sticking the sections on slides with albumen, to immerse the preparations in a mixture of equal parts of a 1 per cent. aqueous solution of safranin and a 1 per cent. aquoso-alcoholic solution of malachite-green for 10-20 minutes. The preparations were, after washing in distilled water, placed in 0.5 per cent. oxalic acid for 2-3 minutes, and then in absolute alcohol until they no longer gave off any colour, whereupon they were mounted in xylol-balsam. By this method the parasites became green and the tissue elements red.

HESSERT, W.-A Simple Stain for Ciliated Bacteria.

Chicago Med. Recorder, 1894, pp. 240-2.

## (5) Mounting, including Slides, Preservative Fluids, &c.

New Method for Securing Paraffin Sections to the Slide or Coverglass.<sup>†</sup>—Miss Agnes M. Claypole finds that "Among the many steps to be taken in making microscopical preparations, that of securing the sections to the slide may seem of minor importance, yet the possibility of ultimate successful results depends largely on the complete reliance to be placed upon the process by which this step is accomplished. Especially is this true of serial sectioning when the disarrangement of the sections renders the slide almost worthless. In Lee's compilation of microscopical methods, 'The Microtomist's Vade-Mecum,' there are about a dozen different processes given for fixing paraffin sections to the slide. These processes fall into two natural divisions, those fitted for material stained *in toto*, and those fitted for sections to be stained on the slide. Of those belonging to the second group, only a few admit of the use of both watery and alcoholic stains, and in most of them heat is an essential part of the process.

Many of the methods involve a previous coating of the slide with a substance that has to dry and be again moistened before the sections can be arranged upon it, such as collodion, shellac, or a gum preparation.

\* Centralbl. f. Bakteriol. u. Parasitenk., 1to Abt., xvii. (1895) p. 631.

† Proc. Amer. Micr. Soc., xvi. (1894) pp. 65-7. 1895

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Some are useful for temporary slides, while in others the intricacy of the process greatly increases the chance of error, and adds to the time required for the work.

There are no methods given in Lee's work of an earlier date than 1880. One of the oldest is the shellac method, now no longer used. Schällibaum's collodion also is best fitted for bulk-stained objects. A slide is coated with a thin even layer of one part of collodion to three or four volumes of clove or lavender oil. The sections are arranged and the slides heated over a water-bath for five to ten minutes, or over a lamp for a shorter time, till the oil has evaporated. Gage and Summers use a pure collodion coat on the slide which is rendered adhesive by clove oil or ether-alcohol. There are many gum methods, but some forbid the use of watery fluids, and others are not fitted for alkaline stains.

Lee recommends Mayer's albumen for use with sections that have to be stained on the slide, and says that he has found it to be absolutely reliable. There is no need to describe so well known a method, the principle is the coagulation of a thin layer of albumen by the use of heat. It is just at this point that the element of uncertainty comes into the process; much heat will injure the tissue, and in avoiding this danger there is a great probability of applying too little heat to coagulate the albumen.

Among other methods given by Lee is one recommended by Strasser.\* It consists of coating the slides thinly and evenly with a mixture of two parts of collodion with one of castor oil—the percentage of the collodion is not given. Sections are arranged on those prepared slides and coated with a thicker solution—collodion concentratum duplex, 2–3 parts; castor oil, 2 parts; no warming is required, but the slide is put direct into a bath of turpentine for two to ten hours to dissolve out the paraffin.

While working during the past year with serial sections, great trouble was experienced with Mayer's albumen method, and after some experimentation, the following plan was adopted. A layer of Mayer's albumen was spread on the slide and the sections arranged. Then a wash of  $\frac{3}{4}$  per cent. collodion was spread over the surface evenly with a camel's hair brush. This coat is allowed to dry, which takes place in about one minute, but a longer time does no harm; practically, one slide dries while the next is being prepared. During the drying many small air bubbles appear, the presence of which indicates the right degree of dryness; these do not cause any inconvenience, as they disappear during the subsequent processes. When dry the slide is put, without heating, into a jar of xylol or benzin for half an hour or more, to dissolve the paraffin. A stay of several hours in the liquid will not injure the tissue. The paraffin may be removed in three to five minutes by constantly moving the slide in the benzin. The benzin or xylol is removed by 95 per cent. alcohol, and the sections are then stained and mounted as desired.

It was found best to have the liquid for removing the paraffin as fresh as possible, or else the thin film of collodion retained a sufficient amount of it to render the surface greasy; benzin was tried, and proved

\* Zeitschr. f. wiss. Mikr., iv. I. (1887) p. 45.

in every way as satisfactory as xylol. Owing to its cheapness it is possible to use benzin in much larger quantities than xylol, and the requisite degree of freshness is easily obtained.

Many slides were prepared without the preliminary coating with albumen, and in all cases the collodion coat was sufficient to keep the sections fastened to the slide, but owing to the well-known uncertainty in making a film of collodion adhere to glass, the albumen was used as a safeguard against failure, the alcohol in the collodion serving to coagulate the albumen. Different per cent. solutions of collodion were tried, and mixtures varying in the proportions of ether and alcohol. No difference was found in the results given by the various mixtures, but the 3/4 per cent. solution was the most satisfactory.

The chief advantage of this method is that it dispenses with the need for an alcohol lamp; an important, and, in the hands of the inexperienced, a somewhat dangerous adjunct of the laboratory is thus removed from constant use. The greatest disadvantage is that, as in all collodion methods, the collodion is liable to take the stain and refuse to give up the colour to treatment. Practically, however, in using the ordinary hæmatoxylins, eosin, picric alcohol, &c., there is no difficulty; it is only with the stronger stains that trouble is found. The use of this thin coat of collodion is a simple and effective method for general histological purposes."

Preparing Liquidambar for Mounting.<sup>\*</sup>—Prof. H. G. Piffard propares liquidambar for mounting in the following manner. A pound of the crude gum (an exudation from Liquidambar styraciftua L.) is liquefied in a water-bath, and then filtered through a Plantamour's funnel lined with two or three thicknesses of cheese cloth. When cold, one pound of xylol is added. The bottle containing the mixture should be shaken several times daily for at least two weeks. It is then filtered two or three times through absorbent cotton or Swedish paper. The thin transparent filtrate is next inspissated at a gentle heat until it has the consistence of molasses. Two parts of this should be mixed with three parts of monobromid of naphthalin and heated gently. The result is a clear amber-coloured fluid, the refractive index of which should be brought to 1.625 by the addition of either of the ingredients as may be required. Preparations mounted in this medium are quite permanent.

Zenker's Fluid as a Fixative.<sup>†</sup>—Dr. A. Mercier records his experience of this fluid, the formula for which is as follows:—Sublimate, 5.0; bichromate of potash, 2.5; sulphate of soda, 1; acetic acid, 5.0; distilled water,  $100 \cdot 0$ . The acetic acid should be added to the rest of the fluid just before using, 10 ccm. to 200 ccm. of the mixture, and a large quantity is to be used, about fifty times the bulk of the piece or organ. The objects used were fowls' embryos from the second to the seventh day, organs of some mammals (cats, bats, rabbits), and worms, young frogs, and salamanders. The smaller objects were immersed for 24 hours, the larger for 48 hours. They were then washed for 6 hours in running water; then 6 hours in 50 per cent. spirit, changed thrice; then 6 hours in 70 per cent. spirit, once changed; then 2–3 days in 90 per cent.

\* Med. Record N.Y., May 4, 1895, p. 547.

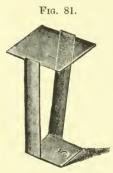
† Zeitschr. f. wiss. Mikr., xi. (1895) pp. 471-8.

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spirit, changed twice or thrice. After this, for 10-15 days in 90 per cent. spirit, containing 1/2-3/4 per cent. tincture of iodine some tincture being occasionally added. Finally, in 90 per cent. spirit until all the iodine was removed. After this the objects were immersed in absolute alcohol, or stained en masse, or soaked in chloroform, &c., preparatory to imbedding for sectioning. The results from this method of fixing are said to be very satisfactory, and the staining with most dyes very good.

Simple Cover-glass Holder.\* - Dr. O. Zoth shows how a simple cover-glass holder can be made from a strip of brass or platinum foil.



The strip of foil, 7 cm. long and 4 mm. broad, widens out to 1 cm. sq. in the centre (see fig. 81) where it is bent twice almost at right angles, and the free ends are provided with two grooves cut by means of a file. The thickness of the foil should be from 0.6 to 0.3 mm., so that it can be easily cut with an ordinary pair of scissors.

#### (6) Miscellaneous.

Improvements in Microscopical Technique.<sup>†</sup> -Prof. H. G. Piffard discusses some of the improvements in microscopical technique, among which he mentions the use of the electric light supplied from the street main. In order to use this it is necessary to employ a Carpenter's

rheostat, which not only effects a uniform resistance and voltage, but affords critical illumination. The author has had made for him lenses specially corrected for the D line of the spectrum, and these are advantageous both in visual and photomicrographic work. The usefulness of monobromide of naphthalin as an immersion medium is referred to, and the author has had constructed for him, by H. R. Spencer of Buffalo, a monobromide immersion lens which works most satisfactorily, and also an achromatic condenser with N.A. 1.40, at about half the cost of English condensers. After showing the advantages to be derived from using lenses and condensers of high aperture, the author proceeds to discuss various mounting media, the preference being given to liquidambar, the formula for which we give elsewhere. Clarifying media, such as the essential oils, are next referred to, and the important fact is pointed out that what is sold as oil of origanum is not such, but in all probability red oil of thyme. The author advises a thorough re-study of our clearing media.

Gaseous Formic Aldehyde for Disinfecting Purposes.<sup>‡</sup>-MM. R. Cambier and A. Brochet report the results obtained by using their apparatus for disinfecting places with gaseous formic aldehyde. The experiments were of two kinds, theoretical and practical, the former carried on under laboratory conditions, the latter in a room of 75 cubic metres  $(5 \text{ m.} \times 5 \text{ m.} \times 3 \text{ m.}, \text{ with two doors and three windows}).$ In

- \* Zeitschr. f. wiss. Mikr., xi. (1894) p. 149.
  † Medical Record N.Y., 1895, pp. 545-9.
  ‡ Ann. de Microgr., vii. (1895) pp. 89-102.

the laboratory series, where the vessels could be hermetically closed, the results were eminently satisfactory, and much like those seen in the testimonials of a new antiseptic; all the germs were destroyed. Although not obtaining absolutely perfect results in disinfecting the room, the value of the method was sufficiently demonstrated, for dust in layers of 1 cm. thick, placed in little pots at various heights in a cupboard, was found perfectly sterilised in 20 hours.

Optimum Temperature for Incubation.<sup>\*</sup>—Mr. Féré comes to the conclusion that 38° is the temperature at which the smallest number of abnormal developments takes place during the first few days of incubation of hens' eggs. By exposing eggs to fumes of alcohol the author finds that injurious effects are overcome afterwards in a large percentage of cases if the eggs are incubated at 38° rather than any other.

Apparatus for making Gaseous Formic Aldehyde.<sup>†</sup> — MM. R. Cambier and A. Brochet have constructed an apparatus for making gaseous formic aldehyde, the object of which is to disinfect rooms, &c., by diffusing therein a definite quantity of this vapour. Its construction is based on the incomplete oxidation of methylic alcohol in contact with air and incandescent platinum,  $CH_3OH + O = H_2O + CH_2O$ . It is composed of a copper vessel to hold the spirit, on which are screwed a number of burners. Each of the burners is composed of a metal tube containing a cotton or asbestos wick, and is surmounted by a cone of platinum wirework, fixed by means of a mica ring. The quantity of air is regulated by a device similar to that of a Bunsen's burner, and the draught kept up by a long glass chimney.

To set the apparatus going, the level of the spirit in the burners must first be adjusted to 1 cm. below the upper edge of the tube, and the holes in the regulator closed. The platinum case is then made red-hot by applying a match to it, the chimney is then put on and the regulatorholes opened.

The best temperature for the platinum is when it is cherry-red; if the flame be too lively the spirit undergoes total conversion into  $H_2O$ and  $CO_2$ ; if too dull, CO as well as  $CH_2O$  is produced. It is advisable to avoid the production of CO, for though it is not found in quantity sufficient to be lethal, yet the blood-spectrum shows the characteristic absorption change. With eight burners 800–1000 grm. of spirit can be converted per hour.

\* Journ. de l'Anat., July 1894. See Amer. Natural., xxix. (1895) p. 62.

† Ann. de Micrographie, vi. (1894) pp. 539-42 (2 figs.).

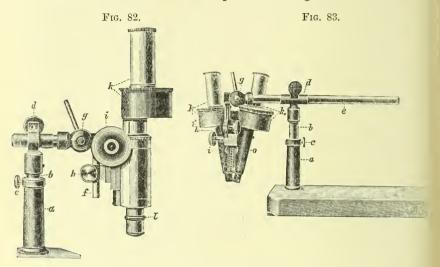
## MICROSCOPY.

## a. Instruments, Accessories, &c.\*

## (1) Stands.

New Preparation Microscope.<sup>†</sup>—Drs. H. Braus and L. Drüner have devised a new form of preparation Microscope which has been made for them by the firm of Zeiss.

The first instrument made is represented in fig. 82. For base it



has a heavy rectangular metal plate (see fig. 82). Screwed into this is a hollow upright a in which a metal rod b, 13 cm. long, slides vertically, and can be clamped at any height by means of the screw c. This rod carries a socket, with clamping screw d, which clasps a second horizontal rod e (compare fig. 83), 28 cm. long, with the end of which a short metal rod f is connected by means of the joint g. The optical instrument is attached to the rod f by a second clamping socket h.

The Microscope consists of a short body-tube (9 cm.) provided with a rack and pinion i. The inversion of the image is effected by an inverting eye-piece k.

The instrument has the following advantages over the Brücke lens:-

(1) A fine and certain adjustment by the pinion which is in close proximity to the hand.

(2) The coarse-adjustment is possible in all directions after loosening the fixing screws.

\* This subdivision contains (1) Stands; (2) Eye-pieces and Objectives; (3) Illuminating and other Apparatus; (4) Photomicrography; (5) Microscopical Optics and Manipulation; (6) Miscellaneous.

† Jena. Zeitschr. f. Naturw., xxix. (1895) pp. 435-42.

(3) The rotation about the axis of the vertical rod b allows the instrument to be moved aside for the purpose of observing the preparation with the naked eve.

(4) The length of the tube causes the head to be at a greater and more convenient distance from the preparation.

Instead of the monocular Microscope, the authors have used with much more advantage a binocular of the form shown in fig. 83. The two tubes, converging on a point about 25 cm. from the eye of the observer, are cast in one piece out of alumi-The two objectives nium bronze. have a special holder so that they can be adjusted to suit a difference in the eyes. The binocular can if necessary be converted into a monocular by removing the two objectives and attaching another by means of the connecting piece m, as seen in

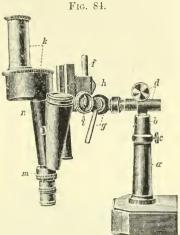


fig. 84. The axis of the single tube n is then adjusted parallel to the rack by means of a rotating disc.

## (3) Illuminating and other Apparatus.

New Hot-Stage with Regulation for Constant Temperature.\*— Dr. W. Behrens has devised a hot-stage in which the regulation of the temperature depends upon an entirely new principle.

The new stage consists of a metal box (fig. 85) which can be firmly screwed to the Microscope stage by the two clamping screws k. In the cover in the centre is a hole through which the objective projects. On one side of this is a narrow slit closed by a glass plate through which can be seen the thermometer t placed in the interior of the box, while at c and b are openings closed by metal caps.

The base-plate has a sufficiently broad piece cut out of it, so that it only rests upon the Microscope stage at the front and back, and leaves a hollow space in the middle in which the preparation can be inserted. On the left side face of the box is the inlet tube a provided with a cock h, and opposite to it on the right-hand side is the exit tube d. Both tubes are movable inwards and can be fixed in position by the clamping screws s and s'. The exit tube possesses also an adjusting arrangement i with a division like the correction arrangement on objective-systems.

The method of using the apparatus in order to obtain a constant temperature is simple. The reservoir B (fig. 86) which is supplied with water by the funnel D is connected by the bent glass tube E and indiarubber tubing F with the inlet tube a. A piece of tubing G of convenient length is attached to the exit tube d for carrying off the water which has passed through the apparatus. The cock h is opened and water

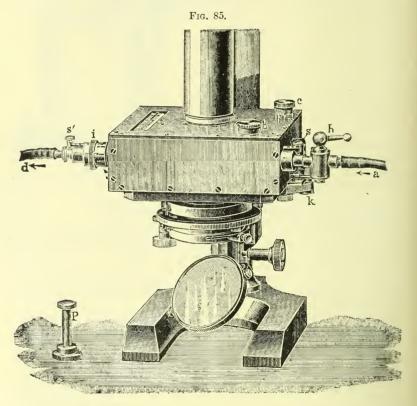
\* Zeitschr. f. wiss. Mikr., xii. (1895) pp. 1-15 (4 figs.).

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drawn from B into A by sucking at G; h is then closed and the box A is filled with water at the ordinary temperature through the opening c. The cap c is then screwed on and the water in B is heated by the Bunsen H to  $60^{\circ}-70^{\circ}$ , as registered by the thermometer T. The cock his again opened a little so that the water only flows from the exit tube drop by drop, and the apparatus is allowed to gradually get warm up to a temperature about  $2^{\circ}$  higher than the temperature required. The cock h is then fully opened and at the same time the tube d is pressed so far inwards that the outflowing water only falls drop by drop. The

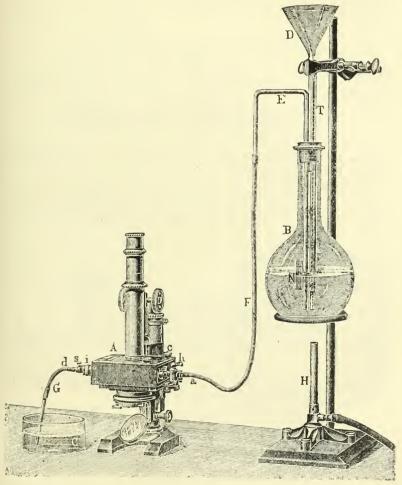


clamping screw s' is then fixed and the apparatus left to itself for a short time and the rise or fall in temperature noted. The tube d is then moved either way through definite intervals until the desired constancy of temperature is obtained.

The means by which this constancy of temperature is obtained appear somewhat complicated. Within the box A, opposite the inlet tube a (fig. 87), lies a metal cylinder g. In the bottom of this is the socket l into which the tube a passes. In front the cylinder g narrows down to the tube m in which is a freely movable piston k. The pistonrod o terminates in a flat plate p which lies exactly opposite to the exit

tube d. The whole arrangement is fastened in the partition wall n which divides the front part of the hot-stage into two parts. The cylinder g is filled with air. As this air expands or contracts on heating or cooling, so does the piston k move forward or backwards. When the piston has moved forward a certain distance its plate p comes in

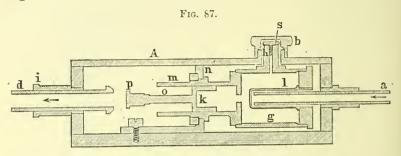
FIG. 86.



contact with the end of the exit tube d and closes it. In that case no water can flow out, and consequently no more hot water can enter. The apparatus then cools by radiation, the volume of air in g diminishes, the piston is drawn back, d opens and water again flows in until the piston once more closes d and so on. In actual practice d is never  $2 \circ 2$ 

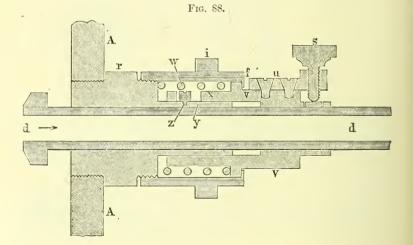
completely closed, but a continual stream of water passes through the apparatus with a varying rate of flow.

The dimensions of the apparatus are such that for a range of temperature from  $+20^{\circ}$  C. to  $+60^{\circ}$  C. the displacement of the piston amounts to 25 mm. For a very exact adjustment of temperature many slight movements of the tube are necessary. An adjusting arrangement



is therefore brought on the tube d, on the division of which, displacements of 0.025 mm. can be read directly. This adjusting arrangement is seen in fig. 88.

By loosening the clamping screw s', the tube d is freely movable in the metal piece r, which has a screw thread on its exterior in which the corresponding screw of the divided head i engages. The projecting



ring f fits over a corresponding ring on the piece v which carries the screw s' for clamping the tube d. Between r and v is a spiral spring w which presses v forwards when the divided head i is turned. The tube y is a prolongation of r, and the tube x a prolongation of v. These two tubes are connected together by the small screw z. By means of the clamping screw s', d is rigidly connected with v and shares in the move-

ments which are communicated to the latter by means of the divided head i. Since the volume of the air in the cylinder g depends not only on the temperature but also on the pressure, it is necessary in order to keep a constant temperature that the level of the water in the reservoir should be kept fairly constant.

The preparation beneath the hot-stage will have a lower temperature than the stage itself. This difference must be determined for different temperatures of the stage by means of a thermometer occupying the position of the preparation. At 30° the difference amounted to  $1^{\circ} \cdot 5$ , and at 60° to 3°.

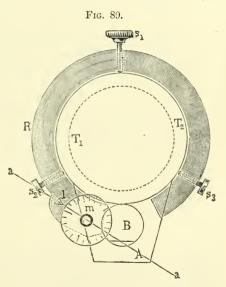
For the perfect air-tight closing of the cylinder g, the piston must be oiled. This oiling can be effected through the neck h after removing the cap b (fig. 87) and unscrewing the screw s. For working at a temperature of 50° the reclosing of h by the screw s can be done at the ordinary temperature, but for work at a temperature of 60° the cylinder must first be cooled to  $+ 10^{\circ}$  before closing h.

Auxiliary Apparatus for the Adjustment with Immersion Objectives.\*—Herr A. van Delden describes a simple arrangement which in difficult cases renders the adjustment with immersion objectives quick and safe.

Round the cuter tube  $T_2$  (fig. 89) is fastened by means of the three screws  $s_1$ ,  $s_2$ , and  $s_3$ , a strong ring which rests above against the edge

of the spring socket in which the inner tube slides. As seen in the figure, the ring is cut through to allow room for the rack B. Through the broader end thus obtained passes a micrometer screw m which is provided with a divided head and long vertical index I (fig. 90). The head has a diameter of about 1 cm., and is divided into 20 divisions. The pitch of the screw is 0.5 mm., and the number of revolutions can be read directly on the vertical index which is divided into half millimetres.

By estimating the tenth of a division on the head, differences up to 0.0025 mm. can be measured. The micrometer screw moves parallel to the rack B, and presses against the column



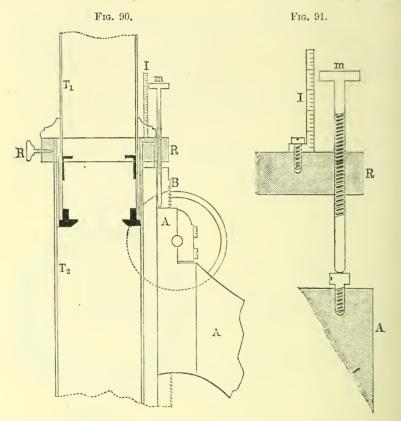
A which is moved by the micrometer screw of the stand (figs. 90 and 91). The apparatus is used in the following way:—Adjustment is first made on a clearly coloured preparation with a fairly strong dry system (e.g. Zeiss D to F), the screw m is then turned until it rests on the

<sup>\*</sup> Zeitschr. f. wiss. Mikr., xii. (1835) pp. 15-18 (3 figs.).

column A, and a reading of its position is taken on the divided head and index.

The dry system is then replaced by the immersion system and as sharp as possible an adjustment is made by means of the coarse-adjustment. The screw m is then turned until it again rests on the column A.

A second reading is taken on the divided head and index, and once for all the difference D is noted between the position of the micrometer screw m in the adjustment with the dry system and in the adjustment with the immersion system. In the observation of any preparation



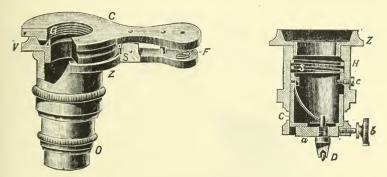
with the immersion system, adjustment is first made with the dry system, the screw m is turned until it touches the column, the body-tube is raised and m again turned through an amount equal to the difference D previously determined. The dry system is then replaced by the immersion system and the body-tube lowered by the coarse-adjustment until the micrometer screw m touches the column A. For the perfectly sharp adjustment only a very slight movement of the ordinary micrometer screw of the stand will then be required. The apparatus is easily detachable from the stand, when not required.

New Object-Finder.\*—Herr R. Fuess has devised a new arrangement for re-finding interesting parts in microscopic objects. The apparatus, represented in natural size in fig. 92, is connected with the objective-changer shown in fig. 93. By its means a circle can be drawn with a diamond point on the cover-glass round any desired part of the object.

To the piece Z, which by its upper conical part is clamped to the objective-holder, is screwed the socket H. In this fits the spring cylinder C which is pressed downwards by the spring S, and carries at

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FIG. 93.



its lower end the sliding piece a, provided with the diamond point D and adjustable in position within a few millimetres. The eccentric position of the diamond point necessary for the production of circles with different radii is effected by the screw b and the counter-spring F.

The little screw c prevents the falling out or rotation of the cylinder C.

In the use of the apparatus, after the desired part of the object has been found by observation, the objective is removed and the apparatus clamped to the objective-changer in its place; the body-tube is then lowered until the diamond-point comes in contact with the cover-glass. The circular line can then be drawn in two ways, either: (1) by rotating the stage, or (2) by rotating the small apparatus in its socket H between the finger and thumb.

The apparatus has the further advantage that by its means lines can be drawn at regular intervals in the case of Microscopes provided with a mechanical stage.

Use of Coloured Light in Microscopy.†—Dr. A. M. Edwards considers that microscopists have not sufficiently appreciated the fact that the clearness with which an object can be seen depends much more upon the character than upon the intensity of the illumination. "Colour is vastly more important than brilliancy." The author gives an account of some experiments which he made on two objectives, 1/5, as long ago as in 1865. With one of these, on *Amphipleura*, Cuba, only lines could

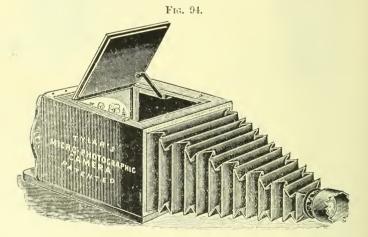
- \* Neues Jahrb. f. Mineralogie, 1895, (1)2 pp., 2 figs.
- † English Mechanic, lxi. (1895) pp. 529-30.

be seen when a blue glass was interposed. With the other, lines could be seen under ordinary conditions, but they disappeared when a yellow glass was interposed or when petroleum light was used instead of daylight. With light which had passed through a flint glass prism, the objectives resolved in the blue but not in any other part of the spectrum. In the blue of the spectrum the author could resolve with a 1/5 what could only be resolved by a 1/15 in ordinary light.

As the result of experiments carried on for several years up to the present time, the author makes use of a slide of mica coloured blue with anilin blue which he places in the path of the illuminating beam of light for the Microscope.

## (4) Photomicrography.

Tylar's Photomicrographic Camera..\*—The author of this little pamphlet of eight pages gives detailed instructions for beginners in the use of the photomicrographic camera which he has devised. The apparatus (fig. 94) may be described as an elementary low-power photo-



micrographic camera. According to the author's instructions it is intended to be used with the Microscepe from which the *eye-piece has* been removed! It is also essential that the whole apparatus should be firmly clamped to the table, and this would almost necessitate the use of the photomicrographic board. This point, however, is not mentioned by the author, so that "the initial outlay of 27s. 6d." does not include an essential part.

The table of exposures for different objectives given by the author is of little use, since the magnification is not stated, and the limits from 3 to 45 seconds are too wide; but the advice which follows is so good that it may be quoted at full :—

"I personally advise the waste of the first plate in obtaining an approximate guide for the exposure of the rest of the plates, and proceed

\* W. Tylar, 'The Art of Photographing Microscopic Objects,' Birmingham, sm. 8vo (n.d.).

thus :—Place the dark slide in position in the camera, taking care before withdrawing the shutter to cut off the light entering the instrument by placing a portion of blackened card between the object and the lens. Now, instead of pulling the shutter out the whole distance, only pull it out 1 inch and expose 3 seconds; replace the card, draw out the shutter another inch and expose 6 seconds; again replace the card and draw out the shutter 1 inch, exprsing 6 seconds; once more repeat the foregoing, again giving 3 seconds only. On developing you will find four ranges of exposures, the last inch having received 3 seconds, the next to it 9 seconds, the next to that 15, and the next, being the first portion exposed, 18 seconds.

"When we develop this plate we shall be able to tell at a glance which part of the plate has had the nearest to the correct exposure, and shall know how to act with the next."

## (5) Microscopical Optics and Manipulation.

Relation of Aperture to the Determination of Minute Structure.<sup>\*</sup> —Mr. C. F. Cox gives vent to some rather heretical remarks concerning the Abbe diffraction theory. The central idea of Prof. Abbe's theory is that in order to obtain a true image of an object, all light-rays from the object must be gathered up by the objective and recombined at its back. The finer the details of structure in the object the greater the dispersion, so that in order to admit all the rays, the aperture of the objective must be proportionately widened. Our finest optical combinations, however, fall far short of an ideal performance in this respect, and, according to Prof. Abbe, no known method of construction can produce an objective capable of giving a true image of details of structure which are closer to each other than within 1/2500 in. It is also the teaching of Prof. Abbe that strictly similar images cannot be expected, except with central illumination with a narrow incident pencil.

The author asks why, if the above statements are true, microscopists continue to use high-power objectives or buy wide-angled substage condensers? He considers that the reason is that they do not really believe the statements; and though they go on repeating the orthodox creed, they qualify it by some secret mental reservation. He confesses that he himself is one of the heretics who still places more or less reliance upon what is seen through the modern high-power objective. Referring to Prof. Abbe's experiments to prove that the diffractionspectra are necessary to the formation of the image, he points out that a progressive reduction of aperture should result in wider and wider divergence from faithfulness in the image, and states that experience does not confirm this inference. In proof of this point he refers to a set of photographs of the Abbe diffraction-plate made by Mr. T. F. Smith, showing the effect of using successively a stop with a 1/12-in., a 1/20-in., and a 1/32-in. opening. Other photographs by Mr. Smith of diatom valves showing the fibrillar structure are also referred to. Photographs of the same diatom valves taken first with an old-time water-immersion 1/12, and then with a recent oil-immersion apochromatic 1/12, were wholly in favour of the wider-angled glass, but the author considers that

\* Journ. New York Micr. Soc., xi. (1895) pp. 74-85.

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this result is due as much to the elimination of spherical and chromatic aberrations in the new objectives as to the mere increase in numerical aperture. The author's position appears to be that an objective with perfect correction will always give truthful images of structures, but that one with narrow aperture will only reveal the coarser structure, while one with wider aperture will correctly image the finer as well as the coarser parts of the structure up to a limit depending on this aperture.

Limit of Microscopical Vision.<sup>\*</sup>—Dr. A. C. Stokes makes some "dogmatic statements" on the limit of the resolving power of the Microscope. The greatest number of lines ever seen through a Microscope is about 120,000 to the inch. The greatest number photographed by Van Heurck with the 1/10 in. objective, 1.63 N.A., using monochromatic blue sunlight, is 127,500 to the inch. This is about the number of the longitudinal lines on *Amphipleura pellucida*. Dr. A. Fock† states that the best Microscopes can resolve, with central illumination, 63,750 divisions to the inch; with oblique illumination 125,000 to the inch; and with the aid of photography 300,000; and these limits are probably too high. The theoretical limit is of course higher. According to Van Heurck's calculation a diamond objective, 2.50 N.A., would theoretically resolve with central illumination and white light 120,000 lines to the inch, with blue light 130,000 to the inch, and by photography 159,000 to the inch; while with oblique light these numbers would be about doubled.

## (6) Miscellaneous.

Introduction to the Study of Rocks under the Microscope.<sup>‡</sup>—This text-book "is intended as a guide to the study of rocks in thin slices" under the Microscope, and, as such, should be of interest to those microscopists who do not wish to restrict their observations to the organic world.

A short introductory chapter contains a few notes on the optical properties of minerals, with instructions on the measurement of extinction angles, observation of the interference colours, pleochroism, &c.; but no systematic account is given of the various physical and chemical methods for the determination of minerals. For these the reader is referred to standard works on the subject. The introduction is therefore followed at once by the systematic description of the various rock types, treated for the most part under the three headings: Constituent minerals; Structure; Leading types. The massive igneous rocks are divided by the author into plutonic, intrusive, and volcanic; and under each of these heads the families are taken in order of increasing basicity.

The sedimentary rocks are dealt with under the tour groups: Arenaceous, Argillaceous, Calcareous, and Pyroclastic. This portion of the book will supply a real want, since in most previous text-books on petrology the massive igneous rocks have alone been considered, e. g. we still wait for the second part of Teall's 'British Petrography.'

Finally the metamorphic rocks are treated under the two divisions of thermal and dynamic metamorphism.

- \* The Observer, vi. (1895) Pract. Micros., pp. 97-100.
- † See this Journal, 1894, p. 395.
- 1 A. Harker, 'Petrology for Students,' Cambridge, 1895, 306 pp., 75 figs.

Brownian Movement.\*—M. C. Maltezos adds a note to his memoir on the Brownian movement which appeared in the 'Annales de Chimie et de Physique' for April 1894. In that memoir he states that if the superficial tension is the same round a corpusele in suspension, its effect will be nil. But this state of equilibrium ceases and the Brownian movement commences in the following cases :—(1) When the body has not the same superficial density throughout its surface; and (2) when the liquid near the body is not pure.

The author refers to a paper by J. A. Bliss † which has caused him to slightly modify his views. In that paper is described a series of observations made in order to discover the causes of the phenomenon of flocculation, i. e. the aggregation into flocks of finely suspended matter in water on the addition of a few drops of acid or solutions of different salts. The phenomenon of flocculation explains the observation made by Stanley Jevons that the Brownian movement is almost stopped by the addition of saline or acid solutions. The author, however, in his observations found that after the addition of the saline solution particles form flocks when they are near to each other, and cease to show the Brownian movement when many unite together; but there are others which simply enlarge themselves and continue to move. This latter phenomenon was explained by the existence, as revealed by a higher power, of finer particles near the larger ones.

As the result of his observations the author concludes that the Brownian movement is a phenomenon of capillarity. For a particle suspended in a liquid he considers the two cases: (1) When the particle is near the bottom of the vessel or near another particle; (2) when it is suspended in the middle of the liquid.

(1) When the particle is near the bottom it is in a liquid which is not homogeneous, for quite near the wall and the bodies supported by it is a capillary liquid atmosphere. As the attraction solid-liquid and wall-liquid is greater (in pure water) than the attraction liquid-liquid and wall-solid, the potential energy is a minimum; if the distance of the particle from the bottom is smaller than the sum of the two radii of molecular action (wall-liquid and particle-liquid), repulsions will result and the Brownian movement will be produced.

The same thing should take place when the particle is near another in the middle of the liquid.

(2) When the particle is far from the walls and other particles, if the liquid were quite homogeneous around it, the particle would not present the Brownian movement; but if the liquid were not homogeneous either by reason of its salinity, or of the presence of the immersed objective, or of the superficial non-homogeneity of the solid, the difference of the superficial tension which would result would suffice to push the solid in one direction or another, i. e. to communicate to it the Brownian movement.

Micrographic Analysis.<sup>‡</sup>—Prof. W. C. Roberts-Austen gives an account of a new branch of investigation, "micro-metallography," or the application of the Microscope to the study of the composition of samples of metals such as iron and steel. Dr. Sorby, in 1864, was one of the first to attempt to develop a method of investigating samples of

\* Comptes Rendus, exxi. (1895) pp. 303-5.

† Physical Rev., ii. (1895) No. 11, ‡ Nature, xlii. (1895) pp. 367-9.

iron and steel by examining polished sections under the Microscope. The progress which has been made in micro-metallography since that time is well shown in a recently published monograph by M. F. Osmond.\* The author explains how the polished section of steel is treated with suitable reagents in order to reveal the five main constituents. He shows how complicated is the structure of ordinary steel, and points out that although a micro-section of a mass of steel closely resembles a rock-section, yet its investigation under the Microscope is rendered much more difficult by reason of the various allotropic forms in which iron itself can occur. Diagrams are given showing the effects of annealing steel. The author considers that the method of microscopic analysis is capable of wide application in metallurgy, and that it should take its place in the ordinary routine of every steelworks laboratory.

The late Frederic Kitton, Hon. F.R.M.S .- We regret to have to record the death, on 22nd July last, of Mr. Frederic Kitton, one of our Honorary Fellows since 1876.

He was the son of Samuel Kitton, and was born at Cambridge on 24th April, 1827. Although placed in business at Norwich before he was 20 years of age, mercantile pursuits were most uncongenial to him, and his natural inclination was in the direction of science, especially the study of the Diatomaceæ, in which he soon distinguished himself.

The following is a list of Mr. Kitton's contributions to the Transactions of the Society :---

- 1873. Prof. Smith's Conspectus of the Diatomaceæ. (Monthly Micr. Journ., ix. pp. 165-7.)
  - Remarks on Aulacodiscus formosus, Omphalopelta versicolor, &c., with Description of a New Species of Navicula. (Op. cit., x. pp. 6–9, pl. xxi.)
  - Description of some New Species of Diatomaceæ. (Op. cit., pp. 205-7, pl. xxxviii.)
- 1874.New Diatoms. (Op. cit., xii. pp. 218–20, pls. lxxxi. and lxxxii.)
- 1875. Number of Striæ on the Diatoms of Möller's Probe-Platte (Op. cit., xiv. pp. 45-6.) Diatomaceæ in Slides of Sta. Monica Deposits. (Op. c., xvi. p. 232.)
- 1876.
- 1877. An Essay on the Classification of the Diatomaceæ. By P. Pettit. Translated by F. Kitton. (Op. cit., xviii. pp. 10-14, 65-77, pls. clxxxvii. and clxxxviii.) New Diatoms from Honduras. Described by A. Grunow. With
  - Notes by F. Kitton. (Tom. cit., pp. 165-86, pls. exciii.-exevi.)
- 1878. On some New Genera and Species of Diatomaceæ. By P. Pettit. Translated by F. Kitton. (Journ. R. Micr. Soc., 1878, pp. 237-45, pls. xiv. and xv.)
- 1879. The Thallus of the Diatomaceae. (Op. cit., 1879, pp. 38-40.) New Species and Varieties of Diatomaceæ from the Caspian Sea. By A. Grunow. Translated with Additional Notes by

F. Kitton. (Tom. cit., pp. 677-91, pl. xxi.) The Diatoms of the London Clay. By W. H. Shrubsole. With

1881. a List of Species, and Remarks. By F. Kitton. (Op. cit., pp. 381–7, pl. v.)

\* Bull, de la Soc. d'Encouragement, x. (1895) p. 460.

**Professor Loven.**—We have lost a notable name from our list of Honorary Fellows by the death of Prof. Sven Loven, who died late in August at the age of 86. Prof. Loven was one of those early zoologists who did not limit themselves to one small branch of the animal kingdom, nor was he of that type of zoologist who confines himself to adult forms. What is now known as the larva of *Polygordius* was discovered by Loven in 1842, and that Lovenian larva, as it is often called, has been perhaps the subject of as interesting speculations and studies as any. In later years Prof. Loven confined himself to the study of Echinoderms, in the investigation of which he was the recognised head and master, and all students of the subject who had the great good fortune to enjoy his personal acquaintance feel a regret for the loss of a friend as well as of a great man of science.

Louis Pasteur.-Great and severe as have been the losses of late in the list of the Honorary Fellows of this Society, a blow greater than all fell on us on September 28th, when one of the greatest geniuses that ever lived, and the greatest benefactor of mankind and of animals, dicd near Paris. Since the death of Darwin there has been no more energetic or powerful intellectual force in the world than that of the great Frenchman, who, it may be said, more than once in his life suffered severe attacks of disease as a result of his arduous labours for mankind. Commencing as a chemist, and making a considerable reputation as a chemist, Pasteur devoted the latter half of his life to the investigation of biological problems. If it cannot be said-and indeed it cannot be said-that he solved the problem of the origin of life, yct he was one of those who brought us nearer to its solution when, by his exquisite experiments, he dealt the final blow at the doctrine of spontaneous generation. No question, perhaps, has excited the interest of men of all degrees and of all ages as much as the phenomena of fermentation. It was left for Pasteur to give a complete explanation and to show that the theories of the chemist, represented by no less a man than Liebig, were utterly erroneous. The discovery of the principle of vital action in fermentation led Pasteur to discuss at large the question of the origins of epidemic diseases in mau and animals, and gave risc to one of the most fertile ideas of modern times, that of preventive medicine. It was one of the first results of his discoveries that Sir Joseph Lister was put in the way of making those experiments on antiseptic surgery which have revolutionised the practice of the surgeon and abated the pains of the patient. To his own countrymen, in three remarkable particulars, Pasteur's investigations have resulted in wealth so extensive that Prof. Tyndall was led to say that the discoveries of Pasteur alone would suffice to pay the ransom required by Germany. Sent by the French Government to investigate the diseases of silkworms, he restored the fortunes of the silk industries of Southern France. Led to investigate the fermentation of beer, he discovered a method of making pure yeast on a large scale; this completely altered the beer industry. The manufacture of the wines of Bordeaux, which had often been uncertain and unequal, was, by the teaching that he was able to give to the winc manufacturer, brought to a high state of improvement. Turning to the cattle of the field, he discovered a method by which that scourge, anthrax, could be successfully combated, and a commission sent to inquire into the results of his methods found that the cattle which he rendered immune did not die: while those which he had not touched died from this fell disease. So successful were his experiments on anthrax, that he turned his attention to what is perhaps the most dreaded of all human diseases, and he was successful in discovering a method by which hydrophobia could be

almost always successfully combated. These are some of the greatest benefits which this great man bestowed not only on France, but on the world; and richer as mankind is by his life's work, the poorer do we feel it to-day to be by his death.

Professor Ryder.-Although Prof. J. A. Ryder was not an Honorary Fellow of this Society, his name has been so frequently of late years mentioned in our abstracts that it is only right that we should express our regret at the death of this distinguished embryologist at the early age of 43. Ryder began to make himself known to biologists when he was embryologist to the United States Fish Com-In 1886 he became Professor of mission. Comparative Embryology at the University of Pennsylvania. The origin of sex, heredity and variation, the evolution of the skeleton, and special subjects, as dynamics in evolution, and the mechanical genesis of the scales of fishes, were some of the many subjects on which he laboured, and on which he published results of great importance.

### $\beta$ . Technique.\*

#### (1) Collecting Objects, including Culture Processes.

Apparatus for Drawing off 10 ccm. of Nutrient Medium.<sup>†</sup>—The apparatus devised by Dr. K. Knauss for removing 10 ccm. of gelatin or bouillon is a cylindrical funnel (fig. 95). The upper part of the outflow tube is closed by a ground glass rod which projects some few cm.

above the top of the funnel, ending in a sort of round handle. The cylindrical part of the funnel is graduated in 10 ccm.'s from 10-60 ccm.

\* This subdivision contains (1) Collecting Objects, including Culture Pro-cesses; (2) Preparing Objects; (3) Cutting, including Imbedding and Microtomes; (4) Staining and Injecting; (5) Mounting, including slides, preservative fluids, &c.; (6) Miscellaneous.

+ Centralbl. f. Bakteriol, u. Parasitenk., 1te Abt., xvii. (1895) pp. 878-9 (1 fig.).

The method of using the apparatus merely consists in raising the glass rod so that 10 ccm. escape, and then lowering it. The end of the pipe is cut off obliquely, and is surrounded by four glass knobs which are intended to prevent the inside of the test-tube, where the cotton-wool plug is to be placed, from becoming contaminated, as it otherwise so frequently is.

Method for Inoculating Agar on Blood-serum.\*-Dr. S. Grosglik has found that test-tubes containing agar or blood-serum can be easily and successfully inoculated by making use of the condensation water. A drop of the fluid to be examined is first inoculated in tubes containing sterilised water, bouillon, or liquid gelatin, and these tubes are then well shaken. From these tubes the condensation water of the culturetubes is in its turn inoculated, and then allowed to run over the surface of the medium. Should the fluid to be examined contain a large number of organisms, the original source must be diluted several times in the usual way before the culture-tube is inoculated. For this procedure the culture-tubes should be wide, and if the condensation water have evaporated, it may be replaced by sterile water. In this way colonies suitable for making pure cultivations can be easily obtained in 24 hours.

Sterilising Blood-serum by means of Porcelain Bougie Filters.†-Dr. P. Miquel, having recently been obliged to prepare large quantities of blood-serum for the bacteriological diagnosis of diphtheria, has found that the porcelain bougie filter satisfactorily disposes of all germs in this fluid, provided that it be free from blood-corpuscles. Filtration is rapidly accomplished, especially at a temperature of 40°-50°.

Cultivation Medium for the Gonococcus.<sup>‡</sup>—Herr Kiefer recommends the following medium for cultivating gonococcus :-- Ascitic fluid which has been filtered and distributed into test-tubes is discontinuously sterilised at 62°. An equal bulk of agar (1/4 of a test-tube) of the following composition is then added :—3.5 per cent. agar, 5 per cent. pepton, 2 per cent. glycerin, 0.5 per cent. salt, and the mixed contents of each tube are poured into a Petri's capsule. It sets in about a minute, and is then ready for use. The cultivation should be carried on at a temperature of 35° · 8-36°.

Bacteriological Examination of Diphtheritic Membrane-§-According to Herr Jakowski, the solution recommended by Roux for staining diphtheria bacilli has no advantage over that of Löffler, and the author never succeeded in staining these organisms by Gram's method. It is also recommended to use three or four test-tubes for inoculations, as the bacilli may develop in pure cultivation in the third, and almost invariably in the fourth.

\* Centralbl. f. Bakteriol. u. Parasitenk., 1te Abt., xvii. (1895) pp. 826-9.

† Ann. de Microgr., vii. (1895) pp. 261-5.

1 Berlin. Klin. Wochenschr., 1895, No. 15. See Centralbl. f. Bakteriol. u. Parasitenk., 1<sup>te</sup> Abt., xvii. (1895) p. 847. § Gazeta lekarska, 1894, p. 1878. See Centralbl. f. Bakteriol. u. Parasitenk.

1<sup>te</sup> Abt., xvii. (1895) pp. 897-8.

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Indol Reaction in Diphtheria Cultures.\*-MM. Palmyrsky and Orlowsky find that old diphtheria cultures (three weeks) give the cholera-red reaction with hydrochloric and sulphuric acids. Young cultures do not give the cholera-red reaction. For the reaction to succeed, the presence of indol and of nitrites is necessary. In young cultures the latter are absent or in such small quantities that, notwithstanding the presence of indol, the reaction fails.

Colour Reaction of Nitrous Acid on Cultivations of Cholera and other Bacteria.†-While the indol reaction is common to many bacteria and is therefore not a specific test, yet according to M. Lounkewitsch the Griess-Islovay reagent possesses the special power of detecting 1 per million of nitrous acid by staining the medium a deep red. The reagent is composed of 0.10 grm. naphthylamin in 20 grm. distilled water and 0.5 grm. sulphanilic acid in 150 grm. acetic acid. The desired effect is produced by adding to the culture one-fifth of its volume of the reagent. The colour is produced with cholera cultures 6-24 hours old, or with cultures of Vibrio metschnikovi 24-48 hours old. The reagent also produces the colour with the coli bacillus and the bacillus of mouse typhoid, but not with the bacillus of Eberth or with the vibrios Finkler-Prior, Müller, Deneke. The reaction recommended by the author and called by him the nitrous acid reaction to distinguish it from the red indol reaction of Bujwid and Dunham, has the following advantages. It may be obtained with cultures 6, 12, 24 hours old, while the indol reaction cannot before 24-48 hours. It manifests itself in a few seconds, while the indol reaction does not appear till after two or more hours. It can be used with both pepton and gelatin cultures. The colour is deep and bright, while that of the indol is quite pale. The cholera red reaction is unable to distinguish the vibrios Finkler-Prior, Deneke, &c., from the cholera vibrio, while the Griess-Islovay reagent can.

Simple Method of Isolating Acid-producing Bacteria.<sup>‡</sup>—In the study of the bacteriology of milk it is often desirable, says Prof. H. W. Conn, to obtain all the acid organisms separate from the alkaline producing species, and this may be done as follows :- An ordinary beefpepton-gelatin solution is made to which is added 3 per cent. milksugar and enough normal litmus solution to give a deep purplish colour. Plates are then made in the ordinary manner. Wherever an acid organism is present it produces acid from the milk-sugar, and the acid turns the litmus red. The result is that after a day or two the plates begin to be dotted over with little red spots, and these when isolated are the acid-producing organisms.

Cultivating Crenothrix polyspora on Solid Media.§-Herr Rössler succeeded in cultivating Crenothrix polyspora on pieces of brick sterilised by heat. To the water was added some sulphate of iron, as the fungus only thrives in ferruginous waters. After some time at the room

† Wratsch, 1895, No. 1. See Ann. de Microgr., vii. (1895) pp. 267-8.

t Mier. Bull., xii. (1895) p. 4.
 § Arch. d. Pharmacie, ccxxxiii. (1895) p. 189. See Centralbl. f. Bakteriol. u. Parasitenk., 1te Abt., xviii. (1895) p. 25.

<sup>\*</sup> Medycyna, 1895. See Ann. de Microgr., vii. (1895) p. 268.

perature, the picces of brick were completely grown over by the Crenothrix.

Tree-stems as Filters.\*-The discovery made by Pfitzer that seawater could be deprived of its salts and rendered drinkable by filtering it through tree-stems has been tested by Herr Wilm on tree-stems of various kinds of wood of 1-0.5 m. long. and 0.4-0.5 m. in circumference. The stems were connected with an air-pump by means of lead tubing. The permeability of the different woods was very unequal, and only a pressure of 3-5 atmospheres could be used, for with more the bark cracked and the water spurted out. The filtered sea water was at first yellowish and opalescent, afterwards becoming bright and clear. It tasted salty from the beginning. The yellow colour was due to the wood juices, which gave the Trommer reaction for sugar. The amount of salt in the first litres was somewhat less than in non-filtered water, though soon it attained the same concentration. The experiments showed that only very large stems could produce salt-free water. As the procedure was tedious and expensive, it cannot be used for practical purposes, because the stems must be large and their bark must be unimpaired, and even then they only deliver salt-free water in small quantities for a comparatively short time, soon getting blocked up and grown up with bacteria.

The suitability of tree-stems for filtering off bacteria was also examined into. Ordinary tap-water was forced through stems of fixed size for 4-5 hours, the water being examined bacteriologically both before and after the experiment. For the first 2-3 days the water was germ-free, but ultimately it came to contain more than the unfiltered. Stems sterilised by forcing boiling water through lost the power of filtering altogether.

Microscopical Plate Counting.<sup>†</sup>—According to Herr Neisser, plate colonies are best counted in the following ways :--(1) Thinly sown plates of pure cultures (less than 600 colonies) are better counted with an ordinary hand lens than with the Microscope. (2) Thickly sown plates (especially water) should be counted with the Microscope. (3) Mixed plates should always be counted with the Microscope on account of the presence of very small colonies.

Quick Method of Filtering Blood-Serum.<sup>‡</sup>-Drs. G. Campbell and A. D. Ghiselin, while disclaiming any originality for their idea, describe a new filter which answers every purpose, and which can be readily prepared at a moderate price. The filter proper is on the principle of a single bougie water filter sufficiently strengthened to allow the safe use of a high pressure, and so arranged that a sterilised flask may be attached to the bougie in such a manner that the filtrate undergoes no risk of contamination. To the filter is connected a drum filled with liquefied carbon dioxide, such as is used in charging soda-water. The drum consists of an iron cylinder 4 ft. long by 4 in. diameter. To the upper

\* Hygien. Rundschau, 1895, pp. 445-50. See Centralbl. f. Bakteriol. u. Parasitenk., 1<sup>te</sup> Abt, xviii. (1895) pp. 26-7. † Zeitschr. f. Hygiene, xx. pp. 119-45.

See Centralbl. f. Bakteriol. u. Parasitenk., xviii. (1895) p. 25.

<sup>‡</sup> Bull. Johns-Hopkins Hospital, vi. (1895) pp. 91-3 (3 figs.). 1895

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end of this cylinder there is fixed a safety valve, and also a valve by which the pressure can be turned on. To this valve is attached a very thick-walled rubber hose. The method of using the filter is as follows:— A rubber-stoppered flask having two tubes passing through the stopper is the vessel used for collecting the filtrate. One tube is short and has its upper end enlarged and loosely packed with cotton. To the outer end of the long tube is attached a piece of best hose about 2 ft. long, divided in the middle, and having the two pieces joined by a glass nozzle. Further details are given concerning this apparatus, and it is stated that the serum prepared by it is perfectly clear, coagulates at exactly the same temperature as unfiltered serum, and the filtration does not appear to have any appreciable effect on any toxin or anti-toxin that may be present. The authors state that 1000 ccm. of such serum can be filtered in five minutes.

#### (2) Preparing Objects.

Study of Cleavage Cells.\*-It is pointed out that the principle of Roux's method is very simple, but the experiments require to be carried out with care. That observer found that the best material for observation was obtained from newly captured frogs at the beginning of the normal period of spawning. The phenomena of cytotropism are seen most readily between cells which are separated from the egg by cutting or tearing in an indifferent fluid, such as the white of a hen's egg or a 0.5 per cent. salt solution. For the experiments 5 to 10 ccm. of freshly prepared white of egg is required each day. This is prepared by filtering through a wad of cotton, and the preparation must be perfectly clear. The egg in the morula or blastula stage is first stripped of its gelatinous envelope and placed on a circular glass plate about 3 cm. in diameter. It is then covered with about five drops of the prepared white of egg, and torn open with two dissecting needles. The circular plate is next placed in a round glass dish with a rim 1 cm. high, containing 10 to 15 drops of water. The purpose of the dish with water is to check the evaporation of the medium in which the egg lies, and thus to guard as far as possible against concentration of and currents in the medium. These protected cells may be kept alive in a suitable medium for one or two days. The preparation should be immediately examined with a low objective. The examination of isolated cells in an uncovered medium has the advantage that we can easily change the position of the cells with needles or other means, but it is necessary to check results by examining preparations covered with a slip. The cover-slip for this purpose must be large enough for at least two of the wax feet, which should be 0.75 mm. high, supporting it, to fall on dry points of the object-plate.

Examination of Retina of Birds.<sup>†</sup>—Prof. A. S. Dogiel used 1/10-1/16 per cent. solution of methylen-blue in studying the retina of pigeon, fowl, falcon, owl, and other birds, and was thereby able to demonstrate clearly the ending of the centrifugal fibres and their relation to the cells of the middle ganglionic layer.

\* Arch. f. Entw. Mech. d. Organismen, i. pp. 44-8. See Amer. Natural., xxix. (1895) pp. 511-12. † Arch. f. Mikr. Anat., xliv. (1895) pp. 622-48 (2 pls.).

Preparation of Retinal Cells of Fishes.\* - Prof. J. A. Ryder described a method which leaves but little to be desired for clearness and histological differentiation with sharpness of detail. Specimens which had been splendidly fixed and preserved were stained in toto in an alcoholic solution of hæmatoxylin, and differentiated in toto in a 1 per cent. solution of potassium bichromate.

Study of Spermatogenesis.<sup>†</sup>-Mr. E. V. Wilcox describes his method of preparing the testes of Cicada and Caloptenus. The testes of the former were killed in Müller's fluid; those of the latter either in hot water, in hot or cold corrosive sublimate, or in chrom-osmic-acetic mixture. Some of the testicular follicles of Cicada were stained in Grenacher's alcoholic borax-carmine, others according to Bizzozero's modification of Gram's method. Good results were obtained by double staining with safranin and victoria-green. Crystals of the latter were dissolved in absolute alcohol or in clove oil. The sections were first stained in safranin for from 10-15 minutes; the excess of staining was quickly washed off in 90 per cent. alcohol, and then a very strong solution of victoria-green in absolute alcohol was applied for one to two minutes. Excess of green was washed out with absolute alcohol. The Caloptenus material was all stained on the slide, and good results were obtained with the safranin and victoria-green method.

Cytoplasm and achromatic nuclear parts were stained green, the chromosomes, nucleolus, and centrosomes red. The safranin and green method was the only one by which the archoplasm was made distinct. If Henneguy's method be used the safranin must not be too much washed out or the sharpness of outlines will be lost. It is best to wash out the mordant very thoroughly before using the stain, for the potassic permanganate makes a precipitate with the safranin which renders the section so muddy as to be nearly useless. The best results were obtained by the use of Heidenhain's method, and the so-called black process proved more serviceable than the blue. For either process the sections should be very thin. They must be firmly affixed to the slide, for the washing is best done by a stream of tap-water allowed to run over it. Three washings are necessary, each of which should be thorough. Simple immersion in water does not do as well.

Investigation of Mesoglea of Alcyonium digitatum.<sup>‡</sup>-Mr. W. L. Brown on putting to himself the question whether this mesoglea contains nucleo-albumen, reflected that, thanks to the method recently introduced by Lilienfeld and Monti, the examination of this question microchemically is quite feasible. Specimens hardened in osmic acid were cut with a freezing microtome, then washed thoroughly and placed in a solution of ammonium molybdate. After being washed for a few seconds in a mixture of ether (9 parts) and water (1 part) they were put into a 20 per cent. ethereal solution of pyrogallic acid. The cells in such specimens were seen under the Microscope to be stained black. but the mesoglea was not.

Demonstrating Tubercle Bacilli in Sputum.§ - Dr. S. Stirling praises Von Ketel's method for detecting tubercle bacilli in sputum, a

- \* Proc. Acad. Nat. Sci. Philad., 1895, p. 161.
- † Bull. Mus. Comp. Zool., xxvii. (1895) pp. 3-5.
- <sup>1</sup> Quart. Journ. Mier. Sci., xxxvii. (1895) p. 393. § Centralbl. f. Bakteriol. u. Parasitenk., 1<sup>16</sup> Abth., xvii. (1895) pp. 874-8.

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method which fulfils several csscntial requirements, i.e. it is easily carried out, it is not injurious physically or chemically to the material to be examined, it is quite safe, and the microscopical picture excellent. The method consists in putting 10 ccm. water, 6 ccm. carbolic acid, and 10-15 ccm. of the sputum into a bottle capable of holding 100 ccm. The mixture is thoroughly shaken, 100 ccm. of water are added, and having been shaken up again is poured into a conical glass vessel. In from 12 hours to a few days the sediment is examined. The sediment is obtained by pouring off the supernatant fluid and removing the deposit with a pipette. Cover-glass preparations will stain easily with a simple alcoholic solution of fuchsin, though, of course, the phenol-fuchsin may be used.

The method is applicable to discharges and fluids of all kinds, e.g. milk, urine, fæces, and is specially suited for staining the preparations by Czaplewski's method.

Pancreatin-digestion of Sputum for Demonstrating Tubercle Bacilli.\*-Equal quantities of sputum and warm water alkalinised with soda are, after having been thoroughly mixed with 0.1-1.0 grm. of pancreatin, placed in an incubator, and in 2-3 hours 0.1-1.0 grm. of pure carbolic acid are added to prevent decomposition. As soon as a sediment has formed the supernatant fluid is removed and fresh alkalinised water added and the mixture incubated anew. The process may be repeated again in order to diminish the bulk of the sediment, and then the deposit is dried on filter-paper and examined. In 24 hours, says Dr. Spengler, the amount of sediment is, as a rule, so small that only a few cover-glasses are required for its examination.

The process does not impair the stainability of the tubercle bacilli unless the digestion of the sputum have been carried on too long.

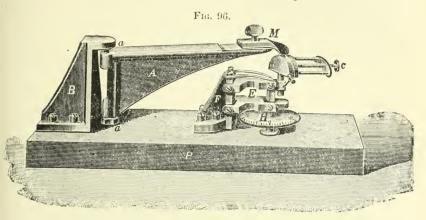
## (3) Cutting, including Imbedding and Microtomes.

Novelties in Microtomes.<sup>†</sup>—Herr B. Pensky gives an account of recent improvements in and new forms of microtomes. Amongst instruments which have been already described in this Journal, he mentions Strasser's ribbon microtome for serial sections, t in which the sections are made to adherc to a paper band; Jung's microtomes; § the "Cambridge rocking microtome"; Minot's microtome; || the Reinhold-Giltay microtome; ¶ and the Reichert large microtome for brain sections.\*\* Besides these hc describes the Fromme microtome (seen in fig. 96), in which the difficulty of production of an exact prismatic slide-way for the knife is obviated by attaching it to the end of a strong arm movable about a vertical axis. The heavy iron base-plate P carries the block B, to which is hinged the arm A, which turns about the point a a and carries at its end the knife M. The raising of the object is effected by a micrometer screw with a divided scale H, the movement of which is transferred to the clamp c by a parallelogram guide E attached to the upright F.

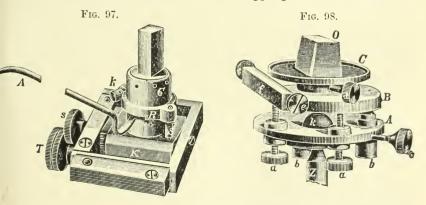
\* Deutsche Med. Wochenschr., 1895, No. 15. See Centralbl. f. Bakteriol. u. Parasitenk., 1te Abt., xvii. (1895) pp. 807-8.

 \* Zeitschr, f. Instrumentenk., xv. (1895) pp. 14–22.
 \* See this Journal, 1891, p. 281, and 1892, p. 703.
 \* Tom. cit., p. 265.
 \* Tom. cit., p. 706. § Op. cit., 1893, p. 264. \*\* Op. cit., 1894, p. 636.

Amongst accessories of the microtome, Mayer and Schoebel's apparatus for raising the object is described. This (fig. 97) consists of a clamping ring R, which, by means of the screw k, is fastened to the cylinder c carrying the object, and is prevented from turning by the pin S, which projects into the block K. The raising is effected by hand



by means of the bent lever, whose shorter arm is set beneath the ring R. The Borgert arrangement for raising the object \* is also mentioned. The Fromme apparatus for the adjustment of the object is seen in fig. 98. The rod Z serves for attaching the holder to the microtome, and carries at its upper end a ball R, which is enclosed by a spherical cap of the plate A and clamped by the screw c. The upper plate B can be rotated



about two axes at right angles by means of the screws a and countersprings b. The plate C is clamped on a pin projecting from the plate B by the screw d, and carries the paraffin block O. For celloidin preparations a boxwood plate can replace the metal plate C. On the plate B is a projecting piece, against the edge f of which the celloidin block

<sup>\*</sup> See this Journal, 1893, p. 801.

is pressed by the screw s of the clamp, after the clamp has been attached by means of the screws e. The double knife made by Walb,\* and the section-stretcher of Prof. Born,† are also described.

Electrical Phenomena of Paraffin Sections.<sup>‡</sup>—Dr. G. C. van Walsem directs attention to certain electrical phenomena which occur when making paraffin ribbon sections. Occasionally, when the strip of section is being removed from the band, it is strongly attracted to any larger object, such as the microtome itself, the table, or the hand of the manipulator. The strip may stick or be damaged. This undesirable manifestation of electricity is not derived from the paraffin but is the consequence of the fixation or hardening, especially with Müller's fluid or by Deiter's method. The presence of electricity was demonstrated by means of the gold-leaf electroscope, which also showed that it is negative in quality. It is probably of frictional and not of chemical origin. The electrical phenomena are easily dissipated by the presence of moderate heat; and prevented by exposing the piece to be sectioned to the air of the room for about half an hour previously.

## (4) Staining and Injecting.

Study of Eye of Decapods.§-Mr. G. H. Parker found that the rapid Golgi method, as described by Kölliker, yielded good results when applied to the optic organs of the Crayfish, especially when the preparations, after having once passed through the silver bath, were again put into the solution of osmic acid and potassic bichromate, and then impregnated with silver. A third or even a fourth application of the silver solution often seemed advantageous. On the whole, better results were obtained from material imbedded in paraffin than from that in celloidin. In employing methylen-blue the author followed the general directions given by Retzius. The ganglia must be carefully removed and studied at once, as, soon after the death of the animal, the sharply differentiated blue stain begins to disappear. The author found that the methods of Retzius made it almost impossible to determine the precise location of a ganglionic cell, or the exact direction taken by its nerve-fibre. Since. however, these demonstrations were necessary, he attempted to devise a process for making sections from material stained in this way. In one, an account of which has already appeared, the tissues of the ganglion were fixed and the colour rendered permanent by means of watery corrosive sublimate. The second, or newer method, essentially resembles the first, but the following steps are necessary in employing it. The ganglia, after being freed from the surrounding tissue, must be first put into an aqueous solution of sublimate, then successively into 30, 50, 70, and 95 per cent. of alcohol, each grade, of course, containing its proper proportion of sublimate. The material was allowed to remain in each of these fluids for about a quarter of an hour. From 95 per cent. alcohol it must be transferred for an hour to absolute alcohol containing 8 per cent. sublimate, then for another hour to a mixture of one part of this alcohol to one part xylol, and finally to pure xylol. In this last the preparation may stay indefinitely. Mr. Parker has found a great advantage from the

\* See this Journal, 1894, p. 403.

† Tom. cit., p. 132.

- ‡ Anat. Anzeig., xi. (1895) pp. 41-3.
   § MT. Zool. Stat. Neapel, xii. (1895) pp. 3-7.

use of the method of Vom Rath, in which the tissue is fixed in a solution of osmic, acetic, and picric acids, and platinic chloride, and afterwards reduced in crude pyroligneous acid. It is stated that this method presents the double advantage of being unfailing in its results, and of yielding preparations which are remarkably clear and trustworthy. The author has improved his depignenting fluid (0.1 per cent. aqueous solution of potassic hydrate) by using as a fixative for the sections a mixture of the fixatives of Schällibaum and Mayer. When small drops of each of these fluids are thoroughly mixed on a slide a whitish sticky paste results, which, even in extremely small amounts, resists the loosening action of both potash and absolute alcohol. The number of retinal elements was determined by counting the corneal facets. The author describes in detail the method by which he effected it. In justification of its use he points out that the difference between the estimated and the actual number did not differ by as much as 1 per cent.

Fixing and Staining Nervous Tissue to Demonstrate Changes in the Cells.\*—Dr. G. Mann uses the following fluid for fixing nervous tissue:—Saturated solution of HgCl<sub>2</sub> in 3/4 per cent. NaCl, 100 ccm.; pieric acid, 1 gr.; tannin, 1 gr.; or simply a saturated solution of HgCl<sub>2</sub> in 3/4 per cent. NaCl<sub>2</sub>.

Various stains were used, and the following procedure is given in detail when methyl-blue is used. The ganglia were fixed in HgCl, and imbedded in paraffin. The sections were stuck to the slide by the author's albumen method, and after removal of the paraffin immersed in the following solution :--1 per cent. methyl-blue (water-soluble) 35 ccm., 1 per cent. eosin (water-soluble) 45 ccm., H<sub>2</sub>O 100 ccm. (1) Stain for 24 hours. (2) Remove superfluous stain with water. (3) Dehydrate in absolute alcohol. (4) Place slide in glass vessel containing absolute alcohol 30 ccm. and 1 per cent. NaHO 4 drops, and leave until the dark-blue section has become reddish (1-5 minutes). (5) Wash out all traces of soda with absolute alcohol. (6) Immerse sections in tap-water, and when the bluish-red clouds are no longer given off, transfer to water acidulated with 2-3 drops of acetic acid for 3 minutes. (7) Dehydrate with absolute alcohol and mount in turpentine balsam. If the sections be still too blue, the process must be repeated.

Methylen-blue Staining Granules of Pneumonia and Anthrax.<sup>†</sup>— Dr. N. Pane describes certain granules occurring in cells taken from the blood, spleen, and bone-marrow of rabbits infected with pneumonia and anthrax. These granules stain well with aqueous methylen-blue solution (1:800), and are usually of a decided blue or of a slightly different tone from the rest of the preparation (metachromatism). Similar preparations taken from healthy animals do not exhibit these granules.

After pointing out that these granules resemble those of "Mastzellen," the author negatives their identity by showing that the two sets of granules differ in their receptivity for methylen-blue and dahlia, and in their resistance to a temperature of  $70^{\circ}$ - $75^{\circ}$ . Preparations made from spleen-pulp indicated that the "methylen-blue" granules had a

<sup>\*</sup> Journ. Anat. and Physiol., xxix. (1894) pp. 100-7.

<sup>†</sup> Centraibl. f. Bakteriol. u. Parasitenk., 1<sup>te</sup> Abt., xvii. (1895) pp. 789-94 (3 colrd. figs.).

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bacterial origin, all stages of conversion of the bacteria into granules being demonstrable.

New Methylen-blue Method.\*—Herr A. Bethe has hit upon ammonium molybdate as a suitable substance to combine with the tetramethyldiamidothiodiphenylamin of methylen-blue in order to obtain a less soluble salt than in the ordinary method. Moreover, hyperosmic acid acting on the methylen-blue molybdate in the presence of excess of ammonium molybdate forms a combination darker blue in colour, insoluble in alcohol even after weeks, and with other advantages. For Vertebrates, Bethe recommends the following mixture:—Ammonia molybdate 1 gr., distilled water 10 ccm., peroxide of hydrogen 1 ccm.; hydrochloric acid 1 drop; for Invertebrates, half the quantity of peroxide of hydrogen, and no hydrochloric acid. He gives full details as to the employment of his new method, which has yielded highly satisfactory results.

Modification of Gram's Method: Staining with Thionin.†—After pointing out that the staining of microbes is effected indirectly, as by the Ehrlich and Gram methods, and directly, as by the methylen-blue, with tannin and other methods, M. Nicolle describes a modification of Gram for which the following reagents are necessary:—

Gentian-violet.—Saturated solution of gentian-violet in 95° alcohol, 10 ccm.; 1 per cent. phenol in H<sub>2</sub>O, 100 ccm.

Eosin.—Saturated solution of eosin in  $95^{\circ}$  alcohol, 50 ccm.; alcohol  $95^{\circ}$ , 100 ccm.

Fuchsin.—Saturated solution of fuchsin in  $95^{\circ}$  alcohol, 5 ccm.;  $H_2O$ , 100 ccm.

Orth's Carmine.—To the carmine one-sixth alcohol at 95° is added in order to prevent the sections from becoming separated from the slide.

Pieric Acid Alcohol.—Just sufficient pieric acid is added to 95° spirit as will impart a very pale yellowish tint.

Gram's Iodine.—Iodine, 1 grm.; iodide of potassium, 2 grm.;  $H_2O$ , 200 grm.

Absolute alcohol to which one-third and one-sixth of aceton have been added.

Absolute alcohol.

Alcohol at 95°.

Alcohol and ether in equal parts.

Xylol. Xylol balsam.

To stain cover-glass preparations of a culture, the film is fixed in alcohol-ether and then treated for 4-6 seconds with the phenol gentianviolet, after which it is immersed in the iodine, renewed once or twice, and next placed in the one-third alcohol-aceton.

If a pathological product, it may be double-stained by passing the cover-glass through the eosin solution after decolorising in alcoholaceton. Should the pathological product contain an organism stainable by Gram and another decolorised thereby, the alcoholic solution of fuchsin should be used instead of the eosin.

Sections may be triple-stained by combining the action of carmine,

- \* Arch. f. Mikr. Anat., xliv. (1895) pp. 579-622 (3 pls.).
- † Ann. Inst. Pasteur, ix. (1895) pp. 664-70.

pieric acid, and gentian-violet. After removing the paraffin by means of xylol and the latter with alcohol, the preparation is to be immersed in the alcoholic Orth's carmine for a quarter of an hour. After washing in water it is transferred for 6 seconds to the gentian-violet solution and then to the iodine solution. After decolorising in alcoholaceton (1/3) the preparation is passed rapidly through the pieric acidalcohol, and having been dehydrated in absolute alcohol and cleared up in xylol is mounted in balsam.

The direct method, while applicable to all micro-organisms, should be reserved for those decolorised by Ehrlich or Gram. The necessary reagents are:—Phenol-thionin (saturated solution of thionin in 50° alcohol, 10 ccm.; phenol in  $H_2O$  1 per cent., 100 ccm.); phenol-gentianviolet; alcoholic solution of eosin; alcohol-aceton; absolute alcohol; ether-alcohol, equal volumes; xylol and balsam. Thionin or "violet de Lauth" belongs to the same group as the methylen or toluidin blues, and owing to its slight solubility in absolute alcohol is especially suitable for staining organisms decolorised by Gram.

For cover-glass preparations the thionin or gentian-violet solution may be used, and the cosin employed as a contrast stain in certain cases. For sections the thionin solutions should only be used.

In conclusion, the author says that by adding eosin to Gram's iodine a double staining is effected. Iodine, 1 grm.; iodide of potassium, 2 grm.; saturated solution of aqueous eosin in alcohol at  $90^{\circ}$ , 20 ccm.;  $H_2O$ , 200 ccm.

Methyl-blue and Methylen-blue.<sup>\*</sup>—What follows may be news to some of our readers. "The names of these two substances resemble each other so closely as to cause a greal deal of confusion, especially when an effort is made to abbreviate. We have taken the trouble to get authoritative definitions of these two forms from E. Merck (Darmstadt and New York), and Dr. Grübler (Leipsic).

Methyl-blue is the sodium salt of tri-phenyl-para-rosanilin-sulphonic acid. It is also known as methyl-blue, M.B.I., for cotton. It is a dark-blue powder, forming a blue solution in water, and is used mostly for histological work.

Methylen-blue is a salt of tetra-methylthionin, the double chloride with zinc being the form usually met with, though a simple hydrochlorate, free from zinc, is also in the market, and this is what is supplied when 'chemically pure' is specified. It is used principally for staining in microscopic work, a very extensive use being as a contrast or ground stain for tubercle bacillus, the latter being stained with fuchsin (magenta)."

# (5) Mounting, including Slides, Preservative Fluids, &c.

Formol.†—M. R. Blanchard has a note on this fluid, of which we have heard so much of late. He has himself been using it for leeches. He finds that after a year there is but the faintest alteration in the delicate coloration of these worms. M. Joubin has preserved in it various Cephalopods, and they have retained the same appearance as they have in the living state.

\* Micr. Bulletin, xi. (1894) p. 17.

† Bull, Soc. Zool. France, xx. (1895) p. 93.

# SUMMARY OF CURRENT RESEARCHES RELATING TO

Rapid Method of Making Permanent Specimens.\*-Dr. T. S. Cullen describes a rapid method of making permanent specimens from frozen sections by the use of formalin. Knowing that specimens hardened in this fluid show almost perfect preservation of the cellular structure, it occurred to him that it might be used in the preparation of frozen sections. An excellent permanent specimen may be made in the following way :- The tissue to be examined having been frozen is cut, and the sections placed in 5 per cent. watery-solution of formalin for 3-5 minutes, in 50 per cent. alcohol for 3 minutes, and in absolute for 1 minute. The tissue is now thoroughly hardened and can be treated as an ordinary section, and stained and mounted in the usual way. The blood is lost in frozen sections as a rule, but if the specimens be first fixed in formalin and then frozen, the blood is preserved, although it does not stain very distinctly. Dr. Cullen says that given a piece of tumour from the operating-room it is possible to give as definite a report in 15 minutes as one would be able to give when examining the alcoholic or Müller's fluid specimen at the expiration of two weeks.

New Use of Formic-aldehyde.<sup>†</sup>---MM. Koehler and Lumière describe a new use of formic-aldehyde as a preservative agent for the bodies of mammals. They find that, if doses varying from 50 to 150 ccm. of a one-fifth solution are injected into the digestive tube by the mouth and anus, and into the carotid of a guinea-pig, the animal may be hung up in a dry place and left in the open air for some weeks. In this condition the animal does not become in the least deformed. A specimen which had been thus treated four months earlier was, when exhibited, found to have the tissues absolutely free from putrefaction. The hairs were perfectly intact and the animal had kept its form and was found in a state of preservation which could not be obtained by any other process.

Formalin as a Fixative instead of Osmic Acid.<sup>‡</sup>—Herr A. Durig finds that formalin is more effective than osmic acid in Ramon y Cajal's method; it penetrates deeper, is more certain, and cheaper.

For fixing he used a gradation of 0.5 to 15 per cent. solution of formalin, with variable strengths of potassium bichromate, and thereafter the silver nitrate process.

Formalin as a Fixative.§-Prof. P. Lachi refers to a note by Dr. A. Durig on this subject, and points to the previously published results of experiments made with this method by Prof. Hoyer, Dell' Isola, and himself.

New Method of Preserving Large Specimens. || - Drs. H. Brand and L. Drüner describe the method they employ in the investigation of the nervous system of fish in order to obtain a uniform condition of preservation throughout large specimens. The method consists in introducing the fixing solution through the vascular system.

The fish is chloroformed, the heart exposed and a short glass tube attached to the bulbus aortæ. The glass tube is connected by indiarubber tubing with the flask containing the preserving liquid. A second

- \* Bull. Johns Hopkins Hospital, vi. (1895) p. 67.
- † Bibliograph. Anat., i. (1895) pp. 31 and 2.
- <sup>‡</sup> Anat. Anzeig., x. (1895) pp. 659-60. § Tom. J Jena. Zeitschr. f. Naturw., xxix. (1895) pp. 435-42. § Tom. cit., pp. 790-1.

flask in connection with the water supply serves to keep the constant air-pressure necessary for the injection. A manometer, tube and apparatus for stopping air-bubbles must be inserted between the injection flask and the tube. Since the preserving fluid coagulates the blood, the latter must first be washed away by means of physiological salt solution. The fixing fluid is then introduced. The injection is continued until all visible parts show the change of colour resulting from the effect of the fixing fluid. This is then expelled by water, the vascular system thoroughly rinsed with alcohol, and finally the whole specimen is immersed in alcohol.

Fish treated in the above way exhibit for preparations the following advantages :---

(1) The preservation is perfectly uniform and allows of an exact histological investigation for any given part.

(2) The firmness of the tissue is perfect.

(3) By the removal of the blood the injurious saturation of the tissues with coloured matter of the blood is avoided, and the pure colour resulting from the fixing fluid is obtained.

Mounting Small Objects in Aqueous Media.<sup>\*</sup> — Herr H. Reichelt says that well-dried pollen-grains, fern-seeds, fungus-spores, &c., may be mounted in aqueous media by means of the following procedure. A cover-glass is coated with a thin layer of shellac by just dropping on it some isobutyl alcoholic solution of shellac and allowing it to dry. Upon this film the objects to be mounted are arranged in any manner desired, and then the cover-glass is carefully removed to a space filled with alcohol vapour. For this an ordinary bell-jar or exsiccator answers very well; a few drops of alcohol are poured on the bottom and the preparation placed on a tripod or some convenient vessel. In a few hours the shellac layer will have softened sufficiently to fix the objects to be mounted, and on removal from the alcoholic atmosphere the shellac becomes quite firm again, so that the objects remain in their position while being mounted in watery media.

Study of the Lymphatics of the Mammary Gland.<sup>†</sup>—M. C. Regaud finds that the methods of investigation used by his predecessors are sufficient to explain the divergent results at which they have arrived. He concludes that it is of great importance to preserve the impregnated vessels in a state of distension, but under the influence of strong alcohol, which is generally used to fix pieces treated with silver injection, the impregnated canals empty themselves and contract. The author's professor, M. Renaut, has devised a method which avoids this contraction. He mixes the silver solution with piero-osmic acid, and makes injections with this mixture. The pieric acid is to be employed in a saturated watery solution. It aids in fixing the tissues, and the yellow tinge which it gives to them allows the investigator to judge of the diffusion of the injected liquid. The osmic acid may be from 1 in 300 to 1 in 1000 parts. The nitrate of silver should be a weak solution only, that is, 1 in 400 or 1 in 500. The mixture of these three fluids when dissolved in distilled water leaves no precipitate, and may be preserved for

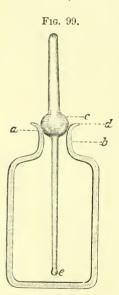
\* Zeitschr. f. angewandte Mikroskopie, i. (1895) pp. 11-2.

† Journ. Anat. et Physiol., xxx. (1894) pp. 719-24.

a long time in vessels of yellow glass, although it is better that it should be made fresh before use. The injection is made either with an ordinary syringe with a fine platinum needle, or by means of an apparatus with continual pressure. When the injection is made, a cube at the centre should be marked off with a razor and placed in strong alcohol. After changing the alcohol as often as necessary, the piece should be cut into sections and these submitted to the action of acetic or formic acid, or other reagents, stained or not, and mounted in glycerin or Canada The author finds that the double action of osmic acid and balsam. nitrate of silver gives excellent preparations. The lymphatics are seen with great distinctness.

Influence of Osmic Acid on the Preservation of Nuclear Structures.\*-Dr. B. Rawitz observed that the nuclei in the peripheral parts of the Salamander's testis, treated with the chrom-osmio-glacial acetic acid mixture, differed from those in the central portion. He interpreted this as a nuclear disruption due to the violent action of the osmic acid. Flemming has denied the justice of this interpretation, Rawitz maintains his position and notes the differences observable when weak and strong solutions are used. But he allows that it is still a case of opinion against opinion.

Criticism of Fixing Methods.<sup>†</sup>—Prof. A. Fischer points out some of the pitfalls of technique. Chemical reaction on the part of the tissue to be fixed, which is not always neutral or alkaline, is apt to give



granular precipitates or coagulations, and these artificial products may often be mistaken for "chromatin" and other formed substances. But his cautions must be read in detail to be rightly appreciated.

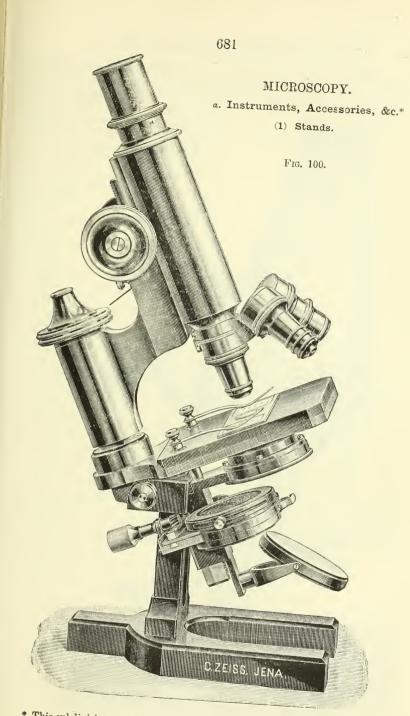
# (6) Miscellaneous.

New Oil-bottle.<sup>‡</sup>—Herr H. Horne has devised an oil-bottle intended for microscopical purposes and so constructed as to prevent the oil from running over. The bottle, made of glass, holds 15-20 ccm. The upper part of the neck is expanded into a funnel-shaped lip d. The stopper is a glass rod with a spherical expansion at the junction of the handle with the dipper. This ball c must be of such size and so carefully fitted that it only just rests on the junction of the lip d with the vertical part b of the neck. The dipper part of the stopper ends in a small ball e.

Geotropic Chamber.§ - Prof. J. Sachs describes a chamber in which the geotropic phenomena in the growth of plants can be observed and recorded without being disturbed by helio-

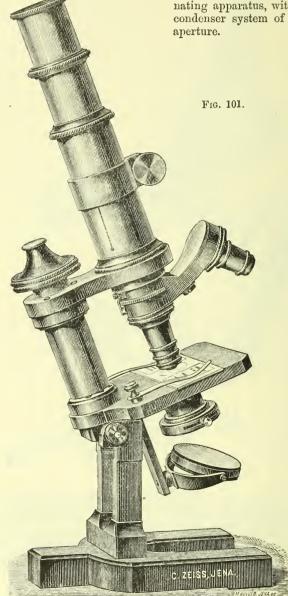
tropism. The image of the growing plant is thrown on to a ruled. glass plate, and observed by a telescope fixed at a distance of 3-4 m.

- † Tom. cit., pp. 769-77. \* Anat. Anzeig., x. (1895) pp. 777-80. † Tom. cit., pp. 769-77. ‡ Centralbl. f. Bakteriol. u. Parasitenk, 1<sup>te</sup> Abt., i. (1895) p. 448 (1 fig.).
- § Flora, lxxx. (1895) pp. 293-302 (3 figs.).



\* This subdivision contains (1) Stands; (2) Eye-pieces and Objectives; (3) Illuminating and other Apparatus; (4) Photomicrography; (5) Microscopical' Optics and Manipulation; (6) Miscellaneous. Zeiss' Stand IVa.\*—This stand is shown in fig. 100 in half full-size. It is inclinable without clamping lever. The vulcanite stage is fixed. The coarse- and fine-adjustment are as in other Zeiss stands. It is

provided with the Abbe illuminating apparatus, with iris and condenser system of 1.20 mm. aperture.



<sup>\*</sup> Zeiss' Catalogue, No. 30, 1895, pp. 46-7.

Zeiss' Stands VI. and VII.\*—Stand VI. (fig. 101) is inclinable and has a fixed stage ( $63 \times 70$  mm.). The coarse-adjustment is by sliding tube, the fine by micrometer-screw.

Stand VII. (fig. 102) is in all respects similar to Stand VI., except that it is not inclinable. It is substantially built for laboratory use.

Great care has been taken in the construction of the fine-adjustment of both these stands, so that they can be used with the highest dry powers, and even with immersion systems if particular care be taken.

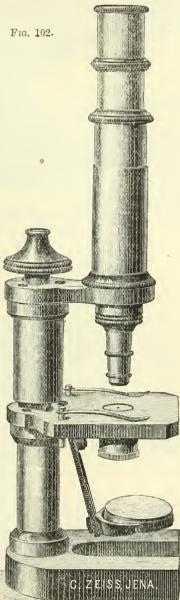
Zeiss' Dissecting Stands.†-The Paul Mayer's Dissecting Stand I., shown in fig. 103, has a heavy horse-shoe foot, and a stage consisting of a large metal frame (10  $\times$ 10 cm.), to which are attached wooden folding hand-rests. The adjustment is by rack and pinion. The dissecting system may be fixed in the ordinary lens-holder p, or in a suitable ring R on the movable arm L R, which is inserted into the ordinary lens-support at L. Bv this latter arrangement the whole of the stage can be scanned.

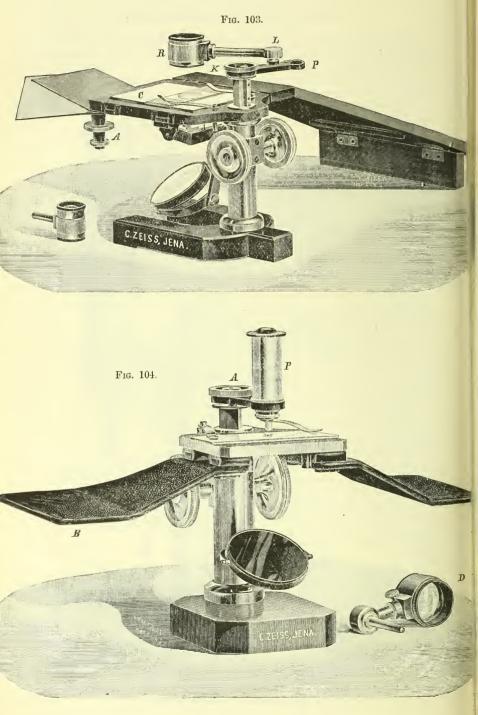
Dissecting Stand III. (fig. 104) has a heavy square metal base, and large stage (75  $\times$  60 mm.) with leather-covered hand-rests B. It is provided with different lens-holders, according to the lenses to be used.

Dissecting Stand IV. (fig. 105) is constructed after the well-known former model of Zeiss. The coarseadjustment is by sliding lens-holder, the fine-adjustment by micrometerscrew.

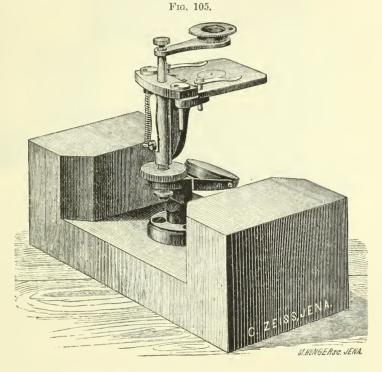
Polarisation Microscope for the Examination of Butter.<sup>‡</sup>—Sig. C. Besana describes a new method of examining the purity of butter, which consists in smearing the butter on a selenite plate so orientated on the Microscope-stage between crossed nicols as to give a reddish-violet field. Pure butter

\* Zeiss' Catalogue, No. 30, 1895, pp. 54-5.
 † Tom. cit., pp. 96-9.
 ‡ Zeitschr. f. Angewandte Mikroskopie, i. (1895) p. 53.





under these conditions shows, according to the author, small grains and globules of fat, all giving the same colour as the field. Margarine, on the other hand, shows on the reddish-violet field grains of a yellow to greenish-blue colour. These colours also occur if the butter is old and raneid or contains boric acid, milk-sugar, or salicylic acid.



Zeiss' Attachable Mechanical Stage.\*—This stage (fig. 106) is in principle similar to those designed by Mayall and later on by Reichert. Two sliding pieces are moved in two rectangular directions by the milled heads S, T, and their position is recorded on millimetre scales. The extent of the rack-and-pinion movement is 30 mm., that of the lateral movement 50 mm.

## (2) Eye-pieces and Objectives.

Zeiss' Achromatic Objectives.<sup>†</sup>—With the aid of the extended list of materials produced by the Jena Glassworks, many of the older types of achromatic lenses have been reconstructed so as to more completely eliminate the spherical and chromatic aberration than was formerly possible.

In fig. 107 the objectives  $a_1$ ,  $a_2$ ,  $a_3$ , are seen attached to the tube and focused with respect to the plane of the object *o o*. They are so mounted

\* Zeiss' Catalogue, No. 30, 1895, p. 95. † Tom. cit., pp. 22-4.

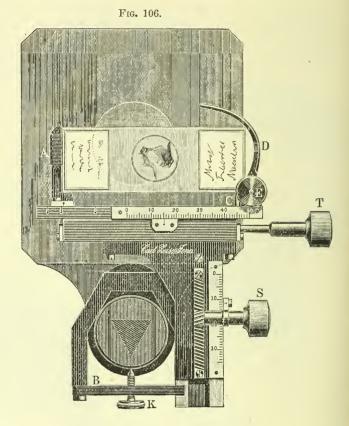
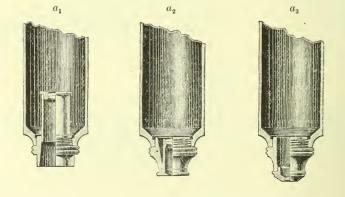


FIG. 107.



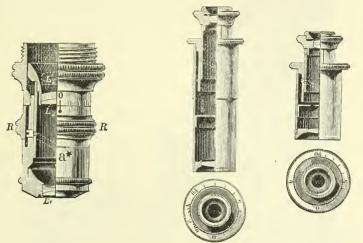
• 0

that, notwithstanding their great focal length, the body of the Microscope remains at its ordinary elevation.

The objective a\* (fig. 108) consists of two achromatic lenses combined after a special formula. The distance between the lenses can be

FIG. 108.

FIG. 109.



varied by the ring R R, like a correction-collar, so that when one of the lower eye-pieces is used the magnification can be varied in the proportion from about 1 to 2.

The new objective D\* is a water-immersion lens of great focal length, but with a relatively small aperture. It is intended for the examination of living zoophytes and plants floating in watertroughs. Fig. 110.

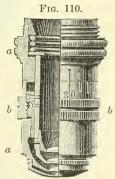
Zeiss' Projection Eye-pieces.\*—These eyepieces (see fig. 109) consist of a collective lens and a compound system which is corrected spherically and chromatically after the principle of apochromatic lenses. A diaphragm is placed between the lenses, and the compound lens can be made to approach it or recede from it.

(3) Illuminating and other Apparatus.

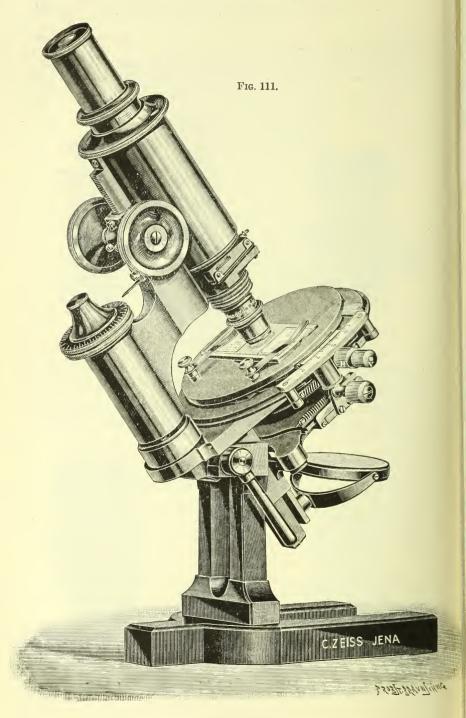
Correction Adjustment.<sup>†</sup>—In fig. 110 is seen the construction of the Zeiss correction arrangement. The ring  $b \ b$  serves to adjust the distance between the two upper double lenses and

the two lower lenses attached to the mounting a a. The divisions on the collar b b indicate for each position of the collar that thickness of cover-glass which gives the best correction for that position.<sup>4</sup> The

\* Zeiss' Catalogue, No. 30, 1895, pp. 20-1.



† Tom. cit., p. 5.



correction for cover-glass thickness is particularly necessary in the case of the apochromatics 4.0 and 3.0 mm. (dry) and 2.5 mm. (waterimmersion).

# (4) Photomicrography.

Zeiss' Photomicrographic Stand.\*-The stand shown in fig. 111 is in size and general arrangement similar to the Zeiss stand Ia. The round rotating stage is of brass, and has a diameter of 100 mm. By means of the co-axial milled heads H and V, it can be moved in two rectangular directions and its position can be read off on the verniers. The body-tube is very short and unusually wide, so as to provide for the use of long focus lenses.

## (5) Microscopical Optics and Manipulation.

Diffraction Theory.<sup>†</sup>—Herr K. Strahl remarks that the knowledge of the diffraction theory is daily becoming more important for the practical optician. It becomes more than ever necessary therefore to understand what is meant by diffraction-theory. According to the author there are two diffraction theories which are constantly being One diffraction theory relates to the modification which confounded. light suffers in passing across sharp edges or between narrow slits.

The diffraction, however, which is of such importance for the telescope, for observation and measurement, is something quite different from this. Here the edges and diaphragms act not as edges consisting of brass or steel, but as geometrical boundaries which prevent the incident lightwave being effective in its whole extent. The greater the effective part, the smaller is the effect of the diffraction.

Compensation of Errors of Objectives.<sup>‡</sup>—Herr K. Strahl gives some of the results which follow from the calculations of the most important errors of objectives which he has made.

According to geometrical optics, it is easy to conceive the possibility of correcting the positive spherical aberration of an objective by the negative equally great one of an eye-piece.

According to geometrical optics, it is, however, impossible to correct the positive error of an objective by an equally great positive error of the eye-piece.

And yet this is, according to the diffraction theory, perfectly possible.

The author, by a closer investigation, finds that the light-distribution in the region of the focus of an objective of which the spherical aberration A is < 1 (for yellow rays this is the case with all newer objectives) is so similar to that of an aplanatic objective, that the difference amounts to scarcely more than one-hundredth of the complete light-intensity. A compensation of such errors is therefore unnecessary.

The author accordingly concludes that the influence of the spherical aberration has been up till now considerably overrated by the theorists.

Indication of Magnification in Micrographic Drawings.§-M. H. Bolsius, referring to Dr. Carazzi's note on this subject, states that he considers that the method advocated in that note should be combined

|| See ante, p. 368.

<sup>\*</sup> Zeiss' Catalogue, No. 30, 1895, pp. 40-1.

Central-Ztg. f. Optik u. Mechanik, xvi. (1895) p. 213. Tom. cit., pp. 183 and 194. § Zool. Anzeig., xviii. (1895) pp. 386-8.

with the old method which consisted in giving the numbers of the eye-piece and objective. In order to judge of the value of a drawing, the indication of the magnification in diameters is not sufficient, at least in the case of high magnifications: in these cases the particular eye-piece and objective used should also be given. In illustration of his contention the author gives two examples in which magnifications of 250 and 370 times can be obtained by four different combinations, in each case, of objectives and eye-pieces of Zeiss.

Theory and Technique of the Microscope.\* — This contribution from the literary remains of Dr. Th. Marsson consists of notes on the theory and technique of the Microscope, arranged under the following headings:—General optical principles; Dioptrics; Lenses; Objective systems; Focal length; Angle of aperture of the system; Eye-piece; Magnification; Field of view; Aberration.

The information conveyed is for the most part such as is given in more extended form in the ordinary text-books, but may be found useful as a review of the more important facts to be remembered in Microscopical work. A few points may be noticed. Under the heading of Objective-systems the influence of the cover-glass is discussed. Divergent rays from the object passing through the cover-glass produce a series of images one above the other, the effect of which is similar to that of spherical aberration. With weak objectives the effect is negligible, but increases with the strength of the objective and with the thickness of the cover-glass. The thickness of the cover-glass also stands in a certain relation to the length of the body-tube: the more the tube is shortened, the thicker must be the cover-glass.

The effect of immersion systems is to give correction of aberration, increase of magnification and of angular aperture, increase of the distance between objective and object, and admissibility of thicker cover-glasses. In the use of the correction arrangement, the Microscope is adjusted on the dark edge of an object, and the lenses are brought nearer together or farther apart, until an equally strong broadening of the edge results, whether the object be brought within or beyond the principal focus.

The objective system should be somewhat over-corrected, the eyepiece a little under-corrected.

The methods of Gray and of Govi for determining the angular aperture are described. In the Govi method the Microscope is in the ordinary vertical position on a dark table. A lens of from 2 to 3 cm. focal length is brought above the eye-piece, and two strips of white paper are placed on the table near the Microscope. These are separated from each other until their images just come on the opposite edges of the field of view. If the distance between the two strips of paper = d, the distance of the objective from the table = a, the tangent of the half angular aperture is

$$\mathbf{T} = \frac{d}{2a}.$$

For testing spherical aberration, a mercury thread in a capillary tube is recommended for weaker objectives, and fine scratches on silvered glass for stronger objectives.

\* Zeitschr. f. Augewandte Mikroskopie, i. (1895) pp. 33-7 and 65-9.

As regards the chromatic aberration, when the distance between objective and object is within the normal focal length, with an over-corrected system the 'image is surrounded by a violet border, passing into blue; while if the objective is beyond the normal focal distance from the object, the image appears surrounded by a red border, passing into yellowish-red. With an under-corrected system exactly the reverse colour-effects are seen. Still more sensitive are bright lines on a dark ground with oblique illumination. A line at right angles to the light falling obliquely from the right, shows with an over-corrected system the left edge violet or blue, the right red or yellowish-red. With an under-corrected system the appearance is just the opposite.

Introduction to Microscopy.\*—This is an excellent book. The optical part is most clearly written, the whole of the chief problems being worked out by examples and diagrams, without a number of formulæ, but yet scientifically. The chapter on the polariscope—not, as a rule, a strong point in the ordinary text-book—while to some extent perhaps recalling Nägeli and Schwendener, is particularly good. The individual apparatus described is, naturally, entirely German, and therefore the weakest part is that devoted to the substage condenser, Abbe's chromatic being the only form dealt with. The practical sections appear equally satisfactory, and altogether it is one of the best works on general microscopy we have met with for some time.

# (6) Miscellaneous.

Determination of the System of Microscopic Crystals.  $\dagger$ —Dr. J. L. C. Schroeder van der Kolk describes the method he employs for determining the system of small crystals under the Microscope. The greatest difficulty is experienced in the case of minute needles with straight extinction. For such cases the author makes use of a glass hemisphere which rests with its convex surface in the opening of the Microscope-stage, while the flat surface serves as stage for the object. The radius of the opening in the Microscope-stage amounts to about 9 mm., that of the hemisphere to about 15 mm. The hemisphere can now be turned in any way required, while the middle point remains stationary.

The solution to be examined is allowed to crystallise on a thin cover-glass which is attached to the glass stage with some oil or Canada balsam. The needle to be examined is now centered and brought into coincidence with one of the cross wires, when it can be turned

- (1) about its own axis;
- (2) about the horizontal normal to its own axis;
- (3) about both axes successively;
- (4) about the vertical.

The author then gives some practical examples of the uses of the hemisphere in distinguishing between isotropic and uniaxial pinakoidal plates, optically uniaxial and biaxial needles, rhombic and monoclinic pyroxenes, and in determining the oblique extinctions of the plagioclases.

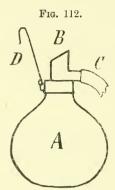
\* Dr. A. Zimmermann, 'Das Mikroskop. Ein Leitfaden d. wissenschaftlichen Mikroskopie,' Leipzig and London, 1895, Svo, 334 pp., 231 figs.

† Zeitschr. f. wiss. Mikr., xii. (1895) pp. 188-92.

2 z 2

# β. Technique.\*

Notes on Experimental Technique.<sup>†</sup> — Dr. E. Centanni uses an apparatus for injecting animals (fig. 112), which consists of an ordinary rubber bulb A. Into the opening is fitted a metal tube B, having a diameter of about 1 cm. It projects about 2 cm., and its end is cut off obliquely. From the side of the tube, just where it leaves the bulb, another metal tube C comes off at right angles. Its diameter is rather less than the former's, and it is connected with a rubber tube about 50 cm. long. D is a hook to attach the apparatus to the operator's coat. When a squirting action is required the opening B is first closed with the thumb and then the rubber ball squeezed. If aspiration be needed the procedure is reversed. If it be necessary to keep up a permanent pressure or aspiration a Mohr's pinchcock is placed on the rubber tube and only opened when the bulb is working. The diameter of the bulb may be



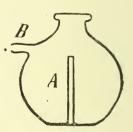


FIG. 113.

from 3-6 cm., and if thick fluids are being worked with, e. g. glycerin extracts, or emulsions of tissues, the wall of the bulb must be very thick.

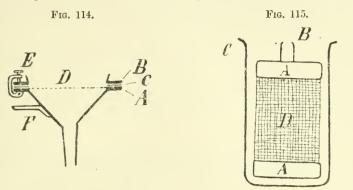
For collecting serum the author uses an almost spherical bottle (fig. 113) with short neck and flat bottom. From the side, at about 2/3 of the height of the bottle, comes off a short tube B. From the middle of the bottom rises up the glass rod A to 2/3 of the height of the bottle. After the apparatus has been sterilised, the free end of the tube in the jugular vein of an animal is passed through the neck of the bottle and blood allowed to flow in until it reaches the level of the side tube B. When the serum has properly separated it is removed through the side tube, the central glass rod preventing the clot from becoming detached.

For obtaining thin emulsions or for separating the solid and liquid portions of emulsions, the author has devised two instruments which filter by aspiration and can be worked with a metal net and with filter

\* This subdivision contains (1) Collecting Objects, including Culture Processes; (2) Preparing Objects; (3) Cutting, including Imbedding and Microtomes; (4) Staining and Injecting; (5) Mounting, including slides, preservative fluids, &c.; (6) Miscellaneous.

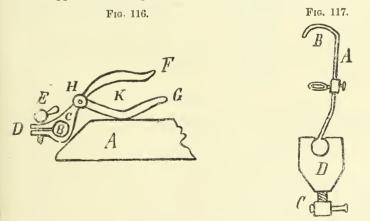
† Centralbl. f. Bakteriol. u. Parasitenk., 1<sup>10</sup> Abt., xviii. (1895) pp. 276-82 (6 figs.).

paper. The funnel-filter (fig. 114) is an ordinary copper funnel along the edge of which a flat ring of tinned iron A is fastened. This is in two parts, the outer being fixed, while the inner one is movable and serves for the support of a circular metal net D, which closes the opening of the funnel. Upon this lies another flat ring B, and the two are fastened



together by four clamps E, and in order to hermetically close the space a rubber band C is interposed. F is a projection or handle for fastening the apparatus to a support.

The bougie-filter (fig. 115) is somewhat like the porcelain bougiefilter in appearance, though shorter and wider. It is made of metal net-



work. The top and bottom as well as a strip 2 cm. broad at each end are made of metal plate and are exactly alike. In order to work these filters the ordinary aspiration apparatus is used. The tube of the funnel is placed in a Kitasato's bottle, and the bougie-filter is connected with a similar receiver by a rubber tube fastened on the nozzle B. A thin emulsion is obtained by placing the pounded up material on the metal net D (fig. 114), constantly stirring during aspiration, or by placing it in a beaker C (fig. 115) with the bougie-filter inside.

For securing the legs of rabbits the author substitutes for the ordinary fastening a more secure contrivance (fig. 116). To the obliquelycut side of the board A is fixed an iron rod B, on which runs the fixation clamp. This consists of the piece D C F which is split on the rod B—D and fixed by the clamp E. At H is a joint on which moves the piece G, the lower fang of the forceps. As G on its anterior half is split in two, the space K can be reduced to nothing, as the two prongs of G allow F to pass between them. There are four of these grips, one for each extremity, and all are covered with rubber. The limb is fastened after it has been passed through K by pressing F down and then screwing up E. For fastening the head the author uses a modification of Tatin's apparatus, which prevents operations on the brain and sometimes on the face, while the apparatus shown in fig. 117 allows of both.

Suggestions on Bacteriological Technique.\*—Dr. A. P. Ohlmacher uses commercial benzene in the technique of bacteriological autopsies on small animals. The benzene is employed as a bath for the instruments and also to disinfect the surface of the animal's body before the final incision. After the skin has been removed the benzene is applied to the surface and then ignited. When the instruments are required they are removed from the dish, the benzene is lighted and allowed to burn off. The instruments should be redipped and ignited as each particular organ is dealt with. This procedure, if properly carried out, satisfies all the requirements of antisepsis.

By far the best methylen-blue is that proposed by Ehrlich for bloodwork, especially for staining *intra vitam* ("methylen-blau nach Ehrlich"). This dye gives much better and more effective results than the pigment ordinarily used.

For staining the diphtheria bacillus methyl-violet 5 B (Grübler) is highly recommended. To a saturated alcoholic solution of the dye, water in the proportion of 10 to 1 is added.

## (1) Collecting Objects, including Culture Processes.

Use of the Microscope in Fermentation Industries, with an Introduction to Study and Cultivation of Yeasts.<sup>†</sup>—The work of Herr P. Lindner is intended for persons engaged in brewing and other fermentation industries and appears to be admirably adapted for their requirements. In it are described the Microscope and its manipulation, the fittings and apparatus requisite for a fermentation-laboratory, their application, the appearances of the different yeasts under cultivation, the method of isolating particular organisms, the sources of contamination and the effects of deleterious organisms, the determination of the number of organisms present in wort, beer, water, &c., and in fact, every branch of the subject that is likely to interest the student of fermentation and the practical brewer.

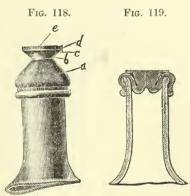
\* New York Med. Journ., lxi. (1895) pp. 268-9.

<sup>†</sup> Berlin, 1895, 4 photographic plates and 105 illustrations. See Bot. Centralbl., 1895, Beih., p. 300.

Obtaining Germ-free Water with Calcium Chloride.\* - Germ-free water, says Herr Bassenge, can be obtained in less than 15 minutes by chemical means. In order to render water which is most impure from the presence of pathogenic bacteria perfectly germ-free, it is sufficient to add 0.0978 grm. of pure calcium chloride (equivalent to about 0.15 grm. of commercial chloride) to the litre, and allow it to act for 10 minutes. The quantity of chloride may be decreased if its action be prolonged, e.g. two hours requires only 0.0108 grm. The chloride not used in the disinfection or in the form of hypochlorous acid is reduced by the addition of bisulphite of calcium, a precipitate of sulphate of lime being deposited. Water thus treated is harmless, has no ill flavour, and is of increased hardness. It can be used for a long time without any influence on the organism (the author has tested it on himself), for the chemical treatment has only imparted to it constituents present in natural drinking waters. No chemical test is required to find out whether all excess of chloride has been reduced; it is quite sufficient to trust to taste and smell.

Air and Germ-tight Cap for Bacteriological Work.<sup>†</sup> -- Herr R. Burri describes an air- and bacterium-tight cap which is adapted for

closing test-tubes or other similar vessels used in bacteriology. One of its chief merits is that it quite prevents diminution in bulk of the medium from evaporation. Fig. 118 shows the shape and position of the rubber cap when placed on the neck of a test-tube or bottle before it is sterilised. At e is a narrow slit which leads to the under part of a. This slit, which can act like a Bunsen valve, is not visible externally, for its edges are firmly approximated. When, however, the contents of the vessel are heated and the air therein expands, the valve opens and allows



the gases to escape. When the pressure inside is equal to that outside the valve closes itself, and as the contents of the vessel cool down the upper part of the cap is sucked in and assumes the shape shown in fig. 119. In this way the vessel becomes hermetically sealed.

Syringe for Bacteriological Purposes.<sup>‡</sup>—Herr K. Ilkewitsch uses a syringe for bacteriological purposes, which consists of a graduated glass pipette a holding 1-5 and 10 ccm. At b is seen the needle fitting into the neck c. To the other end is joined the caoutchouc tube d, 10 cm. long, which runs into the rubber ball e with a diameter of about 5 cm. From the side of the ball projects a short tube made of hard rubber and containing a valve so arranged that it is closed when the ball is pressed

\* Zeitschr. f. Hygiene u. Infektionskrank., xx. p. 227. See Centralbl. f. Bakteriol. u. Parasitenk., <sup>1</sup><sup>to</sup> Abt., xviii. (1895) pp. 239-40. † Centralbl. f. Bakteriol. u. Parasitenk., <sup>2<sup>to</sup></sup> Abt., i. (1895) pp. 627-9 (2 figs.).

<sup>†</sup> Op. cit., 1<sup>te</sup> Abt., xviii. (1895) pp. 55-9 (3 figs.).

together, and open when the ball is free. The illustration shows that the ball e lies between the metal cups g g, from which proceed two

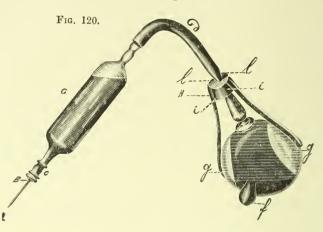
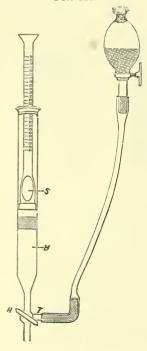


Fig. 121.



handles *i i*. The latter are joined together by a band k running round the rubber tube and playing the part of a hinge. The ends ll of the holders *ii* are flattened, bent at a right angle and embrace the tube d, which is quite closed by their joint action and the elasticity of the ball. Hence by pressing on the cups the tube d is opened and air from the ball passes through the tube. To get the inoculation fluid into the pipette the ball is held in the right hand, the thumb and forefinger nipping the upper end of the pipette or the adjacent part of the rubber By pressing with the other three tube. fingers on the cups air is expelled from The end of the pipette or the the ball. needle is then immersed in the fluid, the thumb of the left hand closing the aperture at f. The pressure is then removed gradually, and the inoculation fluid finds its way into the pipette, and when a sufficient quantity has collected therein the thumb is removed from f. The procedure for injecting an animal is too simple to require description.

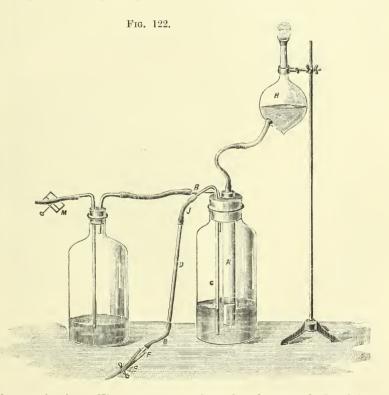
Automatic Burette for Emptying off Sterilised Fluids.\*—Dr. A. Lode describes an apparatus for emptying out definite quan-

tities of fluids such as nutrient media and preventive serum. The special

\* Centralbl. f. Bakteriol. u. Parasitenk., 1te Abt., xviii. (1895) pp. 53-5 (3 figs.).

point of the apparatus is that it works automatically. It consists of a burette (see fig. 121), at the bottom of which is a three-way tap, which connects with the burette, the reservoir, and the outflow tube. Inside the burette is a sort of glass piston-rod, the lower end of which is expanded into a chamber containing a float. The chamber is in communication with the lower part of the burette by a small aperture, so that when the fluid is allowed to flow in from the reservoir the float is pushed up and the further ingress of fluid stayed. By giving another turn to the tap the fluid runs out at the bottom into the vessel placed there to receive it. In this way definite quantities of a nutrient fluid can be safely removed.

Filtering Apparatus for Fluids containing Bacteria and for Preventive Serum.<sup>\*</sup>—Prof. A. Pawlowsky and Dr. G. Gladin have devised an apparatus for filtering fluids, especially anti-diphtheritic serum, which permits any quantity of the filtrate to be drawn off without risk



of contamination. The apparatus consists of a glass vessel closed by a caoutchouc plug with three openings. In one is a Pasteur's bougie A; in another is a short bent tube B, and in the third the glass tube C, the end of which reaches nearly to the bottom of the vessel, while the other

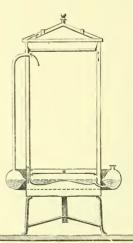
<sup>\*</sup> Centralbl. f. Bakteriol. u. Parasitenk., 1te Abt, xviii. (1895) pp. 170-2 (1 fig.).

is joined to the glass tube D by a rubber tube J. D is connected with a pointed glass tube F, also by a rubber tube E, and the pointed end is inserted into a clamped tube G. The end of the tube B is somewhat constricted and plugged with cotton-wool; this tube is connected by means of a Wolff's bottle with an aspirator. A rubber tube joins the bougie to the flask H, the lower half of which is somewhat funnel-shaped. The neck of the flask is plugged with cotton-wool, and the vessel itself fixed to a stand. The whole apparatus is sterilised in an autoclave, or in a steamer. The autoclave not unfrequently cracks the glass vessels.

During filtration the air is removed from the apparatus, in consequence of which the tubes G, E, J become flat, showing that the apparatus is working properly and that it is air-tight. After the filtration is finished, the aspirator is closed and the receiver disconnected from the Wolff's vessel. The clamp is removed from G to E, and the tube G having been removed, the filtrate can be drawn off. The object of the clamp M is to render aspiration with a closed aspirator possible, for if kept open too long damage may ensue to the apparatus; so that after the air has become sufficiently rarefied the clamp M is fixed on. If the Wolff's bottle be large (3 litres), the apparatus will work with the aspirator shut off for 12–20 hours.

Sterilisation of Water by Ozone.\*—Dr. E. van Ermengem describes the system adopted at Oudshoorn for sterilising the water of the Old Rhine. The results appear to be very satisfactory, for not only are all chemical and bacterial impurities removed, but the physical characters





are greatly improved. The system consists in passing powerful electric currents of high tension through dry filtered air. The ozonised air is then forced or aspirated through vessels containing the water to be sterilised. The source of electricity is a Brush machine with alternating currents, and is driven by a small dynamo. The currents pass to two transformers, where they are converted into high-tension currents. The positive electrodes are made of thin copper or platinum plates, placed parallel to one another, and the current reaches these electrodes after passing through resistance-tubes 0.6 inch high, filled with glycerin. The negative pole is connected with the earth.

Müller-Unkel Steam Steriliser.<sup>†</sup>—The chief merit of this apparatus, according to Dr. R. Blasius, is that it gets into effective working condition in about ten minutes. The accompanying illustration (fig. 123)

shows the simplicity of its construction. It consists of two metal cylinders, the inner one being made of copper with a vaulted bottom. The water reservoir is circular and communicates with the inner cylinder

\* Ann. Inst. Pasteur, ix. (1895) pp. 673-709 (4 figs.).

† Zeitschr. f. Angewandte Mikroskopie, i. (1895) pp. 171-3 (1 fig.).

by means of a narrow pipe. The level of the water above the highest part of the vaulted bottom is never more than 1 cm., and it is owing to this that steam is rapidly and copiously developed. Through the space between the two cylinders circulates hot air, which, if desired, can be let out through an aperture in the lid. The excess of steam passes out through a pipe in the side and condenses in the receiver.

Potato Media for Cultivating the Tubercle Bacillus.\*—Herr Ws. Lubinski prepares nutrient media containing potato for cultivating the tubercle bacillus in the following way. Four varieties were used :— (1) 4 per cent. glycerinised potato-broth; (2) 4 per cent. glycerinised potato-agar; (3) 4 per cent. glycerinised potato-meat-pepton-bouillon; (4) 4 per cent. glycerinised potato-meat-pepton-agar. 1 kg. of clean, finely-chopped potato is boiled in 1500 cem. of water for 3-4 hours on the open fire or in a steamer, and then the acid decoction filtered off. The filtrate, mixed with 4 per cent. glycerin and neutralised, is medium No. 1. By the addition of 1-1.5 per cent. agar to the latter, boiling and filtering, medium No. 2 is obtained. Media Nos. 3 and 4 are made like the ordinary meat-pepton media, except that instead of water the potato decoction is used, that is to say, 500 grm. of finely chopped up meat and 1000 cem. of the potato-broth are, after standing for 24 hours, filtered and mixed with 1 per cent. pepton and  $\frac{1}{2}$  per cent. NaCl; after boiling and so on, 4 per cent. glycerin is added.

These media are also used with an acid reaction by merely omitting the neutralising alkali.

Diphtheria Antitoxin as Culture Medium for the Diphtheria Bacillus.†-Profs. A. E. Wright and D. Semple point out the difficulty which the ordinary practitioner has in making a bacteriological diagnosis of diphtheria. This is due to the fact that blood serum, which constitutes by far the best culture medium for the diphtheria bacillus, is not readily obtained. This difficulty can be easily overcome by the use of the antitoxic serum, which is as good a culture medium for diphtheria as the serum which is derived from a non-immunised animal. A small quantityof the antitoxin should be poured into any clean, small widemouthed bottle. This bottle should be brought into the horizontal position, and the albuminous substances of the serum so coagulated as to adhere to the sides of the bottle. This may be conveniently done by laying the bottle sideways over the mouth of a steaming kettle. Any water of condensation having been poured off, the culture medium is to be allowed to cool down. It is then to be inoculated by passing a stout wire or glass rod, which has been brought in contact with the diphtheritic throat, lightly over its surface. The bottle is to be kept as nearly as possible at a blood temperature, and the microscopical examination may be undertaken after 24 hours. The material for examination may be obtained by lightly scraping the surface of the culture medium with a platinum needle.

**Diphtheria Antitoxic Plasma**.<sup>‡</sup>—Prof. A. E. Wright and Surgeonmajor D. Semple state that the plasma instead of the serum of immunised

<sup>\*</sup> Centralbl. f. Bakteriol. u. Parasitenk., 1te Abt., xviii. (1895) p. 126.

<sup>+</sup> Brit. Med. Journ., 1895, No. 1815, p. 907. ‡ Tom. cit., p. 997.

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animals may be used as an injection material. Besides being equally effective there is a much larger yield of the fluid, often over 65 per cent. of the blood volume. Antitoxin plasma can always be obtained perfectly free from red corpuscles. It is readily prepared by leading off the blood from the horse's vein into a little citrate of soda dissolved in normal salt solution. An addition of 5 grm. of citrate of soda for every 1000 ccm. of blood is all that is required, provided that the tube through which the blood is led into the receiving vessel reaches quite down to the bottom of that vessel, so as to ensure a complete mixture. The easiest method of siphoning off the antitoxic plasma is to use a glass tube bent into a U-shape in such a manner as to leave one limb considerably longer than the other. Near the extremity of the longer limb another glass tube is to be fused on at an acute angle. To this side tube a piece of rubber tubing is to be attached. The other end of the rubber tube is fitted with a mouth-piece plugged with cotton-wool. siphon is thus formed which can be started by exhausting the air in the side tube. A tap or a piece of rubber tube fitted with a pinchcock is fixed at the end of the long limb of the siphon.

Importance of Sugar in Cultivating Media.\*—Dr. Th. Smith, after pointing out that the presence of sugar in cultivation media is often of great importance, inasmuch as a differential diagnosis between two or more species may not be possible if sugar be absent, discusses the relations between sugar, acid-formation, gas-formation, and anaerobiosis. In these connections the most important particulars are that with ordinary meat-broth, the formation of acid and gas are only noticed when sugar is present. The formation of acid keeps pace with the splitting up of the sugar and is common to all anaerobes (potential and essential). The formation of alkali requires the presence of oxygen. Hence both in aerobiosis and in anaerobiosis the one process may mask the other. All gas-forming species produce an explosive gas as well as  $CO_2$ . If at least three kinds of sugar (including muscle sugar) be used, the formation of acid and gas are valuable diagnostic criteria. The division into acid and alkali formers must be given up.

Preparing Clear Agar.<sup>†</sup>—Herr L. Zupnik recommends the following modification of Fraenkel's sedimentation method for obtaining clear agar. Fluid agar is poured into a tall glass vessel placed in a steamer on a water-bath. The fire is gradually let out, and the whole apparatus left till the morning. Next day the solid mass of agar is easily removed, and the turbid portion cut away with a knife.

Another procedure worth mentioning on account of its facility and simplicity consists in adding the requisite quantity of agar powder to perfectly clear meat-bouillon. The liquid is next steamed for an hour, and then filtered in a hot-water funnel through a thin layer of hydrophilous cotton-wool. The cotton-wool is adapted to the shape of the funnel, and then moistened with hot distilled water, any excess of water being pressed out with the finger. The hot agar is at once poured in, and it runs out in a full stream perfectly clear.

\* Centralbl. f. Bakteriol. u. Parasitenk., 1<sup>te</sup> Abt., xviii. (1895) pp. 1–9. † Tom. cit., p. 202.

### (2) Preparing Objects.

Development of Limax.\*-Mr. C. A. Kofoid, in his study of the development of Limax, found the following the most successful method for keeping the animals in captivity. A tin box with proper ventilation is filled to the depth of one inch with clean sand, which forms a suitable substratum for the retention of moisture. On this is laid a sheet of moss, to whose under surface the earth still adheres. The leaves of the common plantain furnish acceptable food, and when this is no longer available, fresh cabbage leaves and apple parings can be used. During the first week of captivity, the slugs furnish eggs in great abundance, but after that time the number diminishes and the quality deteriorates so rapidly that it is imperative that a new colony be secured. Abnormalifies in the living egg show themselves in the early stages by a loose assemblage of the cells, and the increasing opacity of the embryo. Before hardening the embryo, it is necessary to free it from the envelopes and albumen which surround it. By inserting two fine cambric needles in one holder, so that the distance between the points is less than the diameter of the unshelled egg, it is possible to hold the egg between these two needles and pierce it by a third. A quick shear-like cut with the third needle against one of the other two tears open one side of the egg, and allows the albumen and the ovum to escape from the envelopes. As the albumen interferes with section-cutting, and obscures whole preparations, it must be removed entirely. This for a long time presented a most serious obstacle to the author's work. Washing off the albumen with water is a very slow and tedious process, and not always successful. Hypochlorite of soda may indeed be used, but the difficulty of using this lies in the necessity of stopping the action of the hypochlorite before it attacks the ovum. It does indeed free the eggs from the albumen, and does not interfere with staining, but the proportion of eggs destroyed in the process is very great. The method which gave the best results was the following. The living eggs are placed in normal salt solution (0.75 per cent.), in which they are at once shelled. They are then freed from the albumen by washing them in the salt solution, which is dropped upon them from a pipette. The operation is carried on in large glass dishes resembling watch-glasses, but provided with flat polished bottoms, which are placed upon a black tile; this renders the eggs visible to the naked eye. The salt solution dissolves away the albumen, leaving the egg entirely free. It can then be transferred to any desired killing reagent by the use of a capillary glass tube. It is well to shorten the exposure in the salt solution as much as possible, for nuclear conditions are somewhat altered by its action. Eggs which are laid in it for ten minutes have their nuclear membranes much distended, and the chromatin gathered into a homogeneous mass in the centre of the nucleus, surrounded by a clear region of nuclear sap. Excellent results were obtained by subjecting the eggs to the action of Fol's modification of Flemming's mixture for one minute, and then transferring them at once to Orth's picro-carminate of lithium. Rapid decolorisation with 90 per cent. alcohol plus 5 per cent. hydrochloric acid gave very good results. The eggs were studied in the clearing

\* Bull. Mus. Comp. Zool., xxvii. (1895) pp. 37-40.

agent under a cover-glass placed on glass rollers made of bits of capillary tubing. This allows the use of high-power objectives, and the orientation of the embryo in any desired position for a camera drawing.

Methods of Investigating Sponges.\*-Mr. G. Bidder's experiments on the alteration of cells during preservation for histological purposes showed him that the dangers of the imbedding process are modified by very gradual dialysis from alcohol into benzole, and largely guarded against by super-hardening in 1 per cent. osmic acid, and in absolute For osmic acid even sponge tissue requires to be cut into alcohol. the smallest practicable pieces, and repeatedly shaken, or the inner chambers will not be thoroughly hardened. Dialysis from water into absolute alcohol, or from alcohol into benzole, each took from 6 to 12 hours. Mr. Bidder's best preparation was stained in bulk with equal parts of Grenacher's hæmatoxylin and 70 per cent. alcohol, being brought into this solution from 40 per cent. alcohol by four equal changes of strength. No acid was used, and the result was a very valuable overstaining of the collars and iris membranes. It will be found convenient to have in a pipette a thin solution of balsam in chloroform, so that it can be squirted instantly on the sections after removal from the chloroform, to prevent drying before the thicker balsam has time to spread.

Study of Paramæcium.†--Mr. Ryder found that osmic acid and corrosive sublimate gave good results in killing and fixing his material, as both reagents act with such rapidity as to exclude in a large measure the production of artifacts. Staining was done on the slide with hæmatoxylin and Biondi's (= Heidenhain's) mixture. Very good results were also to be got by staining objects in toto.

# (3) Cutting, including Imbedding and Microtomes.

Strasser's Ribbon Microtome.<sup>‡</sup>-Prof. H. Strasser gives detailed instructions for mounting and preserving the serial sections prepared by his so-called "Schnitt-Aufklebe" Microtome.§ For sticking the sections on the paper band the author uses the following mixture :--Gum arabic 80 to 100; water 100; glycerin 10; with the addition of a little carbolic acid. The paraffin sections gummed on the paper with the solution can be numbered and kept for months and years unchanged, until opportunities for their further treatment, colouring, &c., may arise

The staining of the serial sections involves the following operations :-

A. Transformation of the paraffin section into a celloidin section. (1) Xylol bath, 1 hour. (2) Bath of 95 per cent. alcohol,  $\frac{1}{2}$  to 1 hour. (3) Collodionising.

B. Staining. (4) Colouring solution (hæmatoxylin acidified with acetic acid). (5) Bath of Müller's liquid, 5 to 10 minutes, wash in water. (6) Bath of permanganate of potash (1 in 600 water), 5 minutes, wash in water. (7) Bath of differentiating liquid (potassium sulphide

\* Quart. Journ. Mier. Sci., xxxviii. (1895) pp. 38 and 9.

† Proc. Acad. Nat. Sci. Philad., 1895, p. 170.
‡ Zeitschr. f. wiss. Mikr., xii. (1895) pp. 154–68,
§ Sce this Journal, 1891, p. 281; 1892, p. 703.

and oxalic acid), 5 minutes, wash in water. (8) Second staining (e.g. in very dilute neutral carmine, 24 hours), rinse, dry.

C. Freeing from water and preserving. (9) 70 per cent. alcohol with a trace of picric acid, 1 hour. (10) 95 per cent. alcohol,  $\frac{1}{2}$  hour, dry. (11) Plates on the sides of the section covered with filter paper moistened with carbol-xylol.

Then follows a layer of canvas, then the second plate paper, and so Finally the plates are placed in (12) thin resin solution, then in on. (13) thick resin solution. (14) Transfer of the sections to glass slide. (15) Mounting.

For the paraffin imbedding of large objects (brains) the author uses the following processes :- Immersion in 95 per cent. alcohol, then in carbol-xylol from 18 to 22 days, allow to evaporate, place in yellow vaselin, first on and then in the oven at 40° C., up to 8 days, until every trace of xylol has been removed; place in a mixture of paraffin, which melts at  $42^{\circ}$ , and yellow vaselin (the proportions are 4:1, or 3 or 2:1according to the size of the plates), in oven at 40°, one to several days.

The author has introduced modifications in his microtome, so as to make it suitable for celloidin objects. In the large model with double slide-way, the old knife-slide is replaced by a new one, in which the knife, by means of a special holder, can be displaced to right or left in a cross slide-way.

## (4) Staining and Injecting.

Formalin as a Mordant.\*-Dr. A. P. Ohlmacher finds that formalin acts as a powerful mordant with anilin dyes. It may be used in 2-4 per cent. aqueous solution as a preliminary treatment before staining. The cover-glass film is treated for one minute with the solution, washed in water, and then stained in the cold. Or it may be used as a base in the same way as anilin, carbolic acid, &c., are; e.g. formalin-fuchsin may be made by adding 1 grm. of fuchsin dissolved in 10 ccm. of absolute alcohol to 100 ccm. of a 4 per cent. aqueous solution of formalin.

Formalin methylen-blue, which affords a very effective stain, is made by dissolving 1 grm. of methylen-blue (methylen-blue Ehrlich) in 100 ccm. of 4 per cent. formalin.

A curious effect is obtained when safranin (O water-soluble, Grübler) is dissolved to saturation in 4 per cent. formalin. This results in a plasma stain, and when sections are previously coloured with the formalin methylen-blue solution a beautiful double stain results, the blue stain being nuclear.

Staining Yolk-Nucleus of Lumbricus.<sup>†</sup>-Mr. G. N. Calkins arrived at his conclusions concerning the nature of the yolk-nucleus chiefly by micro-chemical reactions with differential staining. The combination stain of Heidenhain's hæmatoxylin and orange makes the chromatin and the yolk-nucleus a blue-black, while the nucleus of the germinal vesicle and the cytoplasm are orange. The Biondi-Ehrlich mixture stains the young yolk-nucleus a bright green, while in the older eggs the disintegrated yolk-nucleus is stained a bright red. As the principal constituents of this mixture are methyl-green and acid fuchsin all doubt

\* Med. News, lxvi. (1895) pp. 184-5.
 † Trans. New York Acad. Sci., 1895, pp. 227 and 8.

in regard to the chemical action of these colours was removed by a solution containing basic fuchsin and acid green. The result was a reversal of the colours; the chromatin and the yolk-nucleus were stained red, and the cytoplasm green. Other differential stains were used, and all gave similar results.

Teichmann's Cold Injection.\*—Prof. J. Kollmann explains the excellencies of this method. The red injecting mass consists of powdered chalk 500 grm., red lead 100 grm., thick linseed oil 80–90 ccm., to which carbon disulphide is added in proportions varying with the calibre of the vessels to be injected. The blue mass consists of zinc white 450 grm., ultramarine 25–30 grm., and boiled linseed oil 60–75 ccm., dissolved in carbon disulphide or sulphuric ether. For injecting the lymph-vessels in white, the mixture is zinc white, thickened linseed oil, and sulphuric ether. The advantages over wax injection are great.

# (5) Mounting, including Slides, Preservative Fluids, &c.

Distortion of Sponge-cells in Preservation.<sup>†</sup>-Mr. G. Bidder gives the results of a few measurements on sponge-cells which may be of interest to those who study histology on preserved materials of other groups as well as of sponges. All the sponges were preserved in osmic acid for one hour; followed by alcohol, benzol, and paraffin, and measurements were made of the collar-cells in six series of sections which may be labelled A to F. In C, D, and F, the change from water into absolute alcohol was effected by dialysis. In all but B, the change from absolute alcohol into benzol was made in the same way. A was the only sponge preserved in . 5 per cent. instead of 1 per cent. osmic acid. It alone was decalcified, it alone was stained in bulk with borax-carmine, and it alone was cut by the ribbon method. The distal expansion and fusion of the collars known as "Sollas's membrane" appeared plentifully in the paraffin sections A, B, C, and F; scarcely at all in D and E, but it was not present in the living sections examined from any of the sponges. It was found that the average cubical contraction of the cells is about one-half of their living dimensions. The best series of sections D and the worst A show respectively the following ratios in their dimensions to those of life.

								I	n Series D.	In Series A.
Collar width		••	••		••		••		•83	•5
Basal width	••		••	••		••	••	••	•88	•7
Height	••	••	• •	••	••	••	••	••	·8	•5
Height of colla									$1 \cdot 0$	$1 \cdot 0$
Deduced ratio of volume of cell to that in life										$\cdot 2$
Deduced mean	line	ar co	ntrac	etion	ratio	)	• •	••	·82	•6

The difference of the best two series of sections from all the others lies in the uniformity of their contractions.

We find, then, two principal phenomena due to the transference of cells through osmic acid, alcohol, and benzole, into paraffin, and finally Canada balsam. (1) There is a reduction in the total volume of the cell, which apparently cannot be avoided, corresponding to a mean linear contraction of about 5 to 4 in the best preparations, and 5 to 3 in the

\* Verh. Anat. Ges., ix. pp. 77-88.

† Quart. Journ. Mier. Sci., xxxviii. (1895) pp. 33-8.

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worst. Secondly, independently of the extent to which this takes place there is generally a change of form. It appears impossible almost entirely to avoid this, but by most methods the rectilinear and angular outlines of life are replaced by pyriform, ovoid, spherical, or even oblate contours in the permanent preparations. It was experimentally shown that the extreme changes of cell form were not produced in alcohol, and further that in some sections of the best series stained on the slide in the ordinary way the cells suffered considerable distortion. The chief engine in distortion appears to be the passage from alcohol into benzole, chloroform, or turpentine, and vice versa. It may be noted that the tendency of all the cells to assume a drop-like form proves that the force which effects their distortion is surface-tension. It does not seem unlikely that the reduction in volume is due to the abstraction of water and soluble matters by alcohol.

Preservation of Mammalian Brains by Formol and Alcohol.\*-Messrs. G. H. Parker and R. Floyd think that the advantages of the employment of formol in hardening the organs of the central nervous system over other reagents employed for similar purposes must be obvious to any one that has used it. A sheep's brain, when placed in a 2 per cent. solution of formol, attains in the course of a week or ten days a remarkable degree of firmness and elasticity, while it preserves in great part its original form and colour. The only important defect is a marked increase in volume. With the object of correcting this defect the authors were led to experiment with other hardening fluids. Alcohol and formol appear to do well, and the following combination was found to meet requirements :- Alcohol 95 per cent., 6 volumes, formol 2 per cent., 4 volumes. The formol employed was that sold by Merck of Darmstadt as formaldehyde 40 per cent. Owing to the rapidity with which this mixture penetrates tissues, the hardening of large, freshly prepared brains necessitates little more than simple immersion. Sheeps' brains prepared in this way retain their original colour and form, and show almost no change in volume.

Disinfectant Action of Formalin.<sup>†</sup>—The experiments made by Dr. G. Burckhard as to the disinfecting action of formalin showed that this fluid has strongly antibacterial properties, though the results did not come up to the expectations formed of it. The animals used were mice, pigeons, and rabbits, and the infecting microbes, anthrax and the bacillus of fowl-cholera. The dead animals were placed on wadding soaked with formalin and covered with a bell-jar. This procedure was found to prevent or considerably retard decomposition. Experiments were also made as to the influence of formalin on the metabolic products of bacteria. For this purpose the bacillus of tetanus was employed. The products of a 10 days' old bouillon culture were passed through a Reichel's filter and injected subcutaneously into mice. It was found that while formalin possesses the power of rendering innocuous the products of *Bacillus tetani*, the quantity required for neutralising the toxin is considerably greater than that used for destroying the toxin of diphtheria.

\* Anat. Anzeig., xi. (1895) pp. 156-8.

† Centralbl. f. Bakteriol. u. Parasitenk., 1<sup>to</sup> Abt., xviii. (1895) pp. 257-64. 1895

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Formol or Formaldehyde.\*-Mr. A. B. Lee points out that the already extensive literature which treats of the uses of formaldehyde is much confused by the inaccurate use of the terms formol, formalin, and formaldehyde. The last is the chemical name of the compound HCOH. Formol is the commercial name given by Schering & Co. to a 40 per cent. solution of this substance in water. Formalin is the commercial name given to the same solution by Meister, Lucius and Brüning. The proper way of stating the strength of solutions is to say formol or formalin diluted with so many volumes of water.

Preservation of Cladocera.<sup>†</sup>—Herr T. Stingelin, after trying various methods for preserving Cladocera, found that it was best to content himself with the exclusive use of a 4 per cent. watery solution of corrosive sublimate. Specimens were at once washed in 65 per cent. alcohol, stained in alum-alcohol for at most three minutes, placed in a 12 per cent. solution of alum, and then washed in water. Hardening was effected in the ordinary ascending grades of concentration of alcohol. The author is well satisfied with the preparations which he got.

Catching and Preserving Medusæ.<sup>‡</sup>-Mr. E. T. Browne states that his latest method for catching Medusæ is to use a long tow-net made of bolting silk with a mesh not exceeding  $\cdot 05$  mm. At the end of the net is fastened a zinc can, instead of the usual glass bottle which generally breaks when the contents are specially wanted. The author prefers to use a small rowing-boat and work very gently against the tide, with just a slight pull on the net. If the Medusæ are not visible at the surface, the net should be sunk about two fathoms, and, if not successful, it should be tried near the bottom. It is well to remember that Medusæ are very delicate, and quickly die if they are crowded together in a small bottle. The following is given as one of the best methods for killing and preserving small specimens. Place the Medusa in a large watch-glass with sufficient water to float in. Add with a pipette about 5 drops of a 2 per cent. solution of cocain, and about 10 minutes later add another 5 to 10 drops. When the Medusa has become motionless and the tentacles expanded, add suddenly a saturated solution of picric acid. It is best to reduce with a pipette the quantity of water in the watch-glass just before adding the picric acid, and to use plenty of the latter. Often when the pieric acid is added a precipitate of cocain is thrown down, which must be removed at once by a pipette, and fresh picric acid added. After being in the acid about half an hour specimens should be gradually transferred to 80 per cent. alcohol. The author strongly recommends the use of the best glass-stoppered bottles and perfectly pure spirit for storing specimens in.

#### (6) Miscellaneous.

Method for Counting Blood-corpuscles.§—Herr P. Domény suggests the following method for counting blood-corpuscles or other bodies suspended in a fluid, such as bacteria, algæ, infusoria, &c. A slight

- \* Anat. Anzeig., xi, (1895) pp. 255 and 6.
- † Rev. Suisse Zool., iii. (1895) p. 165.
   ‡ Proc. and Trans. Liverpool Biol. Soc., ix. (1895) pp. 245 and 6.
- § Zeitschr. f. Angewandte Mikroskopie, i. (1895) pp. 168-71.

scratch is made on the under surface of a cover-glass and on the upper surface of a slide. The fluid to be examined having been placed between the two glasses the scratches are brought the one over the other. The cubical contents of the fluid cylinder are then calculated by first ascertaining the diameter of the field with the system in use. This is done by drawing the apparent size of the field at the stage level, and then measuring it with (say) a pair of compasses. This diameter, divided by the magnification, gives the real size. Then the square of the real diameter multiplied by  $\cdot 7854$  gives the area (m) required. The distance (h) between the two glasses or the height of the cylinder is then ascertained by careful and repeated focusing. Then mh is the solidity of the cylinder. The number of the corpuscles or bodies in the field are then carefully and repeatedly counted and an average struck (p). With these data the number of corpuscles in a cubic centimetre is easily calculated. An ocular micrometer divided into squares facilitates the counting. In examining blood this fluid should be mixed with an equal

bulk of 0.75 per cent. salt solution; if this procedure be adopted it will be necessary to multiply p by 2.

Counting-Plate for' Petri's Capsules.\*-Herr Müller-Unkel has produced a countingplate of black highly polished glass on which are marked four concentric circles and sixteen radii. This form is more convenient than the older apparatus used for counting.

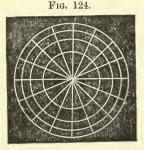
Writing on Metal and Glass.<sup>†</sup> - Herr E. Schöbel recommends the following inks for marking slides, bottles, vessels, &c. :--Silicate of soda 1-2 parts, fluid Chinese ink

1 part, is suitable for slides; while for metal vessels he prefers silicate of soda, Chinese white, 3-4 parts of each, sulphate of baryta 1 part. The mixtures should be kept well corked and shaken before being used.

Demonstrating Bacillus coli communis in Water.<sup>+</sup>—The question<sup>®</sup> whether water which contains Bacillus coli communis is fit for drinking, is, says Dr. Ed. von Freudenreich, an important one. Recent investigations have shown that the microbe occurs in almost every kind of drinking-water, and the demonstration of its presence largely depends on the volume of water used in the test.

Thus, by Vincent's method, wherein about 100 ccm. are used (water 90 ccm., 20 per cent. pepton solution 10 ccm., 7 per cent. carbolic acid 1 ccm., and incubation at  $42^{\circ}$ ) the presence of B. coli can almost always be demonstrated, though if 1 ccm. only be used it may not be detected. Attention is called to the facts that-(1) In chemically bad water (i. e. water containing too much organic matter) wherein bacteria are numerous, Bacillus coli communis is present in abundance. (2) In water containing few bacteria and chemically good, B. coli is present in small numbers only. (3) Very often this organism is absent altogether in water which

3 a 2



<sup>\*</sup> Zeitschr. f. Angewandte Mikroskopie, i. (1895) p. 173 (1 fig.).

<sup>†</sup> Tom. eit., p. 183.
‡ Centralbl. f. Bakteriol. u. Parasitenk., 1<sup>66</sup> Abt., xviii. (1895) pp. 102-5.

is accepted as good. The inferences from the first and last of these three degrees are obvious, while the interest of the second lies in ascertaining whether and to what degree a water is rendered impure by *B. coli*. Mention has already been made of the satisfactory results to be obtained by Vincent's method, but the object is more quickly attained by using bouillon with 5 per cent. milk-sugar.

A number of flasks containing this medium are inoculated with variable quantities of the water to be examined, e. g. with 1, 10, 20 drops, and incubated at 35°. If coli bacteria be present, in 12–24 hours there will be strong fermentation, and this can be rendered more noticeable by shaking the flasks a little. Often water and putrefactive bacteria do not excite fermentation in milk-sugar. When gas is formed the presence of coli bacteria may be safely reckoned on.